IJPSR (2025), Volume 16, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 December 2024; received in revised form, 26 December 2024; accepted, 31 December 2024; published 01 May 2025

A UNIQUE POLYHERBAL FORMULATION FOR COGNITIVE ENHANCEMENT AND MENTAL HEALTH SUPPORT

Shahana Sherin [1], Merin Cleetus [2], Jisha Dev [3], K. Nsakkariya [4] and K. P. Safna Hussan [3, * 5]

J. J. College of Arts and Science, Bharathidasan University [1], Sivapuram, Pudukkottai - 622422, Tamil Nadu, India.
St. Joseph's College [2], Moolamattom, Thodupuzha - 685591, Kerala, India.
Ayurgreen Scientifica Research Institute [3], Ayurgreen Campus, Edappal, Malappuram - 679582, Kerala, India.
Ayurgreen Hospitals [4], Ayurgreen Campus, Edappal, Malappuram - 679582, Kerala, India.
Micro/Nano Technology Center [5], Tokai University, Hiratsuka-shi, Kanagawa 259-1292, Japan.

Keywords:

Polyherbal nervine tonic, LCMS, UV-Visible and FTIR spectroscopy, Antioxidant assays, Molecular docking

Correspondence to Author:
K. P. Safna Hussan

Chief Scientific Officer, Ayurgreen Scientifica Research Institute, Ayurgreen Campus, Edappal, Malappuram - 679582, Kerala India.

E-mail: safnahussain2@gmail.com

ABSTRACT: In an increasingly fast-paced world, the rising prevalence of anxiety and mental health disorders highlights the critical need to preserve cognitive well-being. While mental illnesses often result from complex biological, psychological, and environmental interactions, their precise origins remain elusive. Conventional medicine offers various treatments, but alternative approaches, like nervine tonics, are garnering attention for their natural, therapeutic potential in supporting the nervous system and promoting relaxation. This study introduces *Neuragreen*, a novel polyherbal nervine tonic designed to enhance blood circulation, regenerate nerve cells, and exhibit potent nootropic, anti-Alzheimer, anti-depressant, and antioxidant effects. The formulation blends powerful herbal ingredients, including *Ginkgo biloba*, *Centella asiatica*, *Bacopa monnieri*, *Withania somnifera*, *Mucuna pruriens*, *Pueraria tuberosa*, *Convolvulus pluricaulis*, *Celastrus paniculatus*, Beta carotene, and B-complex vitamins. A multidisciplinary approach was employed, utilizing LCMS, UV-Visible, and FTIR spectroscopy, along with antioxidant assays and molecular docking. LCMS identified diverse bioactive compounds, while UV-Visible spectroscopy revealed the presence of flavonoids, terpenes, and alkaloids. FTIR confirmed the presence of polyphenolic compounds with antioxidant potential, and terpenoids were also detected. Antioxidant assays showed strong free radical scavenging activity with IC_{50} values of 7.3 µg/mL for DPPH and 14.9 µg/mL for SOD. The analytical findings emphasized the formulation's richness in terpenoids, flavonoids, saponins, and alkaloids. Molecular docking studies further supported the formulation's potential nervine activity, identifying nine key phytochemicals with neuroprotective effects, potentially reducing depression and enhancing memory. This research highlights *Neuragreen* as a promising polyherbal formulation with significant therapeutic potential for cognitive and mental health support.

INTRODUCTION: In today's fast-paced world, most people grapple with the challenges of anxiety and mental health disorders. The well-being of one's mind is paramount for leading a fulfilling life. While some turn to medication to manage anxiety and depression, others choose to overlook these issues altogether. It's crucial to recognize that mental health concerns are not trivial matters and should be addressed appropriately. The precise origins of most mental illnesses remain elusive. Researchers have identified that many of these

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.16(5).1000-23

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.16(5).1000-23

conditions stem from a complex interplay of biological, psychological, and environmental factors. Conditions like anxiety, depression, and nervousness can contribute to a state of nervous weakness. Mental health problems encompass a wide spectrum of disorders that influence a person's cognition, emotions, and behaviour. These conditions can have a profound impact on a person's life, affecting their relationships, work, and overall well-being. Some common mental health problems include: a) anxiety disorders, b) mood disorders, c) psychotic disorders, d) personality disorders, e) eating disorders, f) substance abuse disorders, g) post-traumatic stress disorder.

It's crucial to emphasize that mental health issues exhibit a wide spectrum of symptoms, severity levels, and durations. While some individuals may contend with mild symptoms that can be effectively addressed, others may grapple with enduring and incapacitating manifestations. Additionally, mental health challenges can, at times, manifest alongside physical symptoms like fatigue, headaches, and discomfort.

Conventional or Western medicine offers various treatments for mental illnesses. Antidepressants (selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), antianxiety medications (benzodiazepines), antipsychotic medications, mood stabilizers, stimulants (methylphenidate and amphetamine-based medications), antidepressant augmentation agents, and anxiolytics. However, it's crucial to consult with a psychiatrist or mental health professional to determine the appropriate medication and dosage for a specific mental health condition.

Nonetheless, herbal formulations for mental illness have gained attention due to their potential advantages, including their natural approach, potential therapeutic effects, accessibility and affordability, and fewer side effects. According to herbalism, tonic refers to an herb toning the system or a specific organ to strengthen it. A nervine tonic is a type of herbal preparation that is used to nourish, support, and strengthen the central nervous system of a human body. Nervine tonics can be used to improve sleep, reduce anxiety and stress, and enhance overall well-being. They are specifically formulated to restore the balance and tranquility of the body. This tonic helps to keep you feeling energized and active and restores you from stress and anxiety [1]. These nervine tonics are believed to have a calming and balancing effect on the central nervous system. This drug improves blood circulation by opening up blood vessels, regenerates nerve cells, and also possesses potent nootropic activity, anti-Alzheimer, anti-depressant, and anti-oxidant properties [2].

Generally, nervine tonics are metabolized in the liver by enzymes. They help break down the active compound in the herbs into smaller molecules, which can be easily eliminated by the body. Some ingredients in nervine tonics, such as valerian, may be metabolized into compounds that have a sedative effect, while passionflower may be metabolized into compounds that have a calming effect. The metabolism of nervine tonics depends on the metabolic activity of the person's age, sex, body weight, and overall health, as well as the specific ingredients present in the nervine tonic. Most nervine tonics have mild action, and for long-lasting effects, they should be taken over a while [3].

According to naturopathic classifications, many plants used to reinstate balance and tranquility are categorized as nerviness [4]. A nerve tonic may also contain a variety of different herbs that are traditionally used in Ayurvedic, herbal, and naturopathic medicine to promote relaxation and support the nervous system [1]. They are rich in nutrients like calcium, silica, magnesium, B vitamins, and protein. These herbs directly act on the nervous system and improve its functions. Some examples of nervines **Fig. 1** and their reported benefits are as follows:

The concept of using multiple herbs to achieve improved therapeutic results is emphasized in the Ayurvedic text 'Sarangdhar Samhita.' Combining various herbs in a specific ratio can achieve a more significant therapeutic effect while reducing toxicity [5]. The phytochemical constituents of individual plants alone are insufficient to achieve the desired therapeutic effects. Combining multiple herbs and minerals in a carefully calculated ratio can produce a more substantial therapeutic effect

while decreasing toxicity. Polyherbal formulations contain plant-based pharmacological agents that can exhibit synergistic, potentiating, or antagonistic actions due to their diverse active components. These pharmacological principles interact dynamically to produce maximum therapeutic effectiveness with minimal side effects [6]. Combining plants with varying potency, as opposed to using just one plant, has been found to potentially lead to better results due to a phenomenon known as synergism, where the combination of plants produces a more significant effect than the sum of their individual effects. This convenience for patients, as they do not have to take multiple single herbal formulations, improves compliance and therapeutic outcomes. This has made polyherbal formulations more popular in the market than single herbal formulations. Polyherbal formulations are often effective at low doses and safe at high doses, leading to a superior risk-to-benefit ratio. Additionally, polyherbal formulations tend to produce fewer side effects compared to allopathic drugs [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

Herein, a novel polyherbal formulation is introduced as a nervine tonic that is prepared from specified plant parts of *Ginkgo biloba*, *Centella asiatica*, *Bacopa monnieri*, *Withania somnifera*, *Mucuna pruriens*, *Pueraria tuberosa*, *Convolvulus pluricaulis*, *Celastrus paniculatus*, beta-carotene, and B-complex. This drug improves blood circulation by opening blood vessels and regenerating nerve cells. It also possesses potent nootropic activity and anti-alzheimer, anti-depressant, and anti-oxidant properties [2].

As we know, Polyherbal formulations exhibit high efficacy due to active phytochemicals, which may enhance their potency due to the synergetic interaction of active ingredients from different plants. Even though plant products have been consumed since ancient times, the isolated and enriched extracts and their new formulations must be verified to trace the active chemical ingredients and the efficacy of these molecules.

MATERIALS AND METHODS: A nervine tonic, Neuragreen, prepared by the polyherbal formulation of *Bacopa monnieri*, *Withania somnifera*, *Mucuna pruriens*, *Pueraria tuberose*, *Celastrus paniculatus*, *Centella Asiatica*, *Convolvulus pluricaulis*, and *Ginkgo biloba*, is an herbal formulation that has been developed to promote nervous system health and support cognitive function. Each herb in the formulation has a unique set of phytochemicals and medicinal properties, which synergize to provide a wide range of therapeutic benefits. This nervine tonic supports cognitive function, promotes nervous system health, alleviates stress and anxiety, and protects against oxidative stress and inflammation. Neuragreen Tablet by Ayurgreen Ayurveda

Hospitals Pvt Ltd was sourced from Ayush Herbs Pvt Ltd, Himachal Pradesh -176047 India.

Materials: *Bacopa monnieri*, *Withania somnifera*, *Mucuna pruriens*, *Pueraria tuberosa*, *Celastrus paniculatus* were collected from Green Health Herbals and Remedies Pvt. Ltd. Alankode P.O. Kerala, India. *Centella asiatica* and *Convolvulus pluricaulis* were procured from the herbal garden of Ayurgreen Ayurveda Hospitals Pvt. Ltd. Edappal, Malappuram district, Kerala, India. *Ginkgo biloba* was obtained from Ayush Herbs Pvt. Ltd. Nagrota Bagwan, Dist. Kangra and B-complex were obtained from Ayurgreen Ayurveda Hospitals Pvt. Ltd. Edappal, Malappuram district, Kerala, India. The images of all ingredients are depicted in **Fig. 2**.

FIG. 2: MEDICINAL PLANTS AND SUPPLEMENTS USED IN HERBAL FORMULATIONS. (A) *BACOPA MONNIERI* (BRAHMI) - KNOWN FOR COGNITIVE ENHANCEMENT AND MEMORY IMPROVEMENT. (B) *WITHANIA SOMNIFERA* (ASHWAGANDHA) - USED FOR STRESS RELIEF AND VITALITY. (C) *MUCUNA PRURIENS* (VELVET BEAN) - KNOWN FOR ITS NEUROPROTECTIVE AND MOOD-ENHANCING EFFECTS. (D) *PUERARIA TUBEROSE* - TRADITIONALLY USED FOR CARDIOVASCULAR HEALTH. (E) *CELASTRUS PANICULATUS* (INTELLECT TREE) - ENHANCES COGNITIVE FUNCTIONS AND MEMORY. (F) *CENTELLA ASIATICA* (GOTU KOLA) - USED FOR SKIN HEALING AND PROMOTING BRAIN HEALTH. (G) *CONVOLVULUS PLURICAULIS* (SHANKHPUSHPI) - SUPPORTS BRAIN FUNCTION AND REDUCES ANXIETY. (H) *GINKGO BILOBA* - KNOWN FOR IMPROVING CIRCULATION AND COGNITIVE FUNCTION. (I) B-COMPLEX - A SUPPLEMENT THAT SUPPORTS OVERALL HEALTH, INCLUDING BRAIN AND NERVE FUNCTION

Extraction of Herbs: The extraction method used in this study is the magnetic stirrer method with absolute ethanol as the solvent. This method is commonly used for extracting phytochemicals from plant samples as it is efficient and produces high yields of the desired compounds. To begin the interaction process, each medicinal plant is first ground using a pestle and mortar to increase the surface area for extraction. The ground medicinal plants are then weighed and placed in a clean conical flask. Absolute ethanol is added to the conical flask at 1:10 (plant material to solvent ratio) to ensure complete extraction of the phytochemicals. The conical flask is then sealed and placed on a magnetic stirrer, which agitates the mixture overnight to facilitate extraction.

After 24 hours of stirring, the mixture is filtered using filter paper and a funnel to remove any solid particles or impurities. The resulting extract is collected in a clean glass container. The container is placed in a hot oven at 50-60^{o}C to purify the extract further and remove any residual solvent. This temperature is maintained for several hours

until the ethanol completely evaporates, leaving a concentrated crude extract behind. Each crude extract obtained from the different medicinal plants can then be combined to form a polyherbal formulation.

Preparation of Polyherbal Formulation: This study prepared a polyherbal formulation by combining the extracts of different plant materials in specific ratios. The weights of the different plant extracts used were as follows: 375 mg of *Bacopa monnieri* and *Centella asiatica*, 300 mg of *Withania somnifera*, 240 mg of *Gingko Biloba, 225 mg of Pueraria tuberose, Celastrus paniculatus*, and *Convolvulus pluricaulis*, 150 mg of *Mucuna pruriens* and 25.5 mg of B-complex.

The individual plant extracts were first weighed accurately using a digital balance to prepare the polyherbal formulation. The weighed quantities of each plant extract were then combined in a clean container and mixed thoroughly to ensure homogeneity.

Evaluation of the Polyherbal Formulation:

Phytochemical Analysis using LCMS: LC-MS/MS experiments were performed on Agilent 6520 accurate mass MS Q-TOF coupled with Agilent LC 1200. The MS analysis was performed with dual AJS ESI ion source in positive and negative modes. The Agilent molecular ion extraction algorithm did Maas spectral data analysis. The general conditions for mass spectrometry were drying gas (nitrogen) flow 13 L/min; nebulizer pressure 35 psig; drying gas temperature 250ºC; capillary voltage 3500V; fragment or volt 750 V; Oct RF Vpp. A gradient of water (95%) and acetonitrile (5%) was used as the mobile phase for ESI ionization mode at a constant flow of 0.3 ml/min. The mobile phase was fixed as the gradient of acidified methanol (A) and water (B) system for ESI ionization mode. Gradient elution was performed at a constant flow rate of 0.9 ml/min and 1200.00 bar pressure [7].

Spectroscopic Analysis:

UV-VIS Spectroscopy: UV-VIS (Ultraviolet-Visible) spectroscopy is a commonly used analytical technique to identify and quantify phytochemicals in plant extracts. It is based on the absorption of light by molecules, and the absorption pattern can be used to identify and quantify the molecules present in the sample. It is based on the principle of Beer-Lambert law, which states that the absorbance *(A)* of a solution is proportional to the concentration *(c)* of the absorbing species and the path length *(b)* of the light through the solution.

$$A = \varepsilon b c$$

Where ε is the molar absorptivity or extinction coefficient of the absorbing species, a Jasco UV-Visible spectrophotometer model V-550 was used to record the UV-Vis spectra of a polyherbal formulation in ethanol. The baseline was corrected using an ethanol solvent before analysis. The operating conditions used for UV analysis depend on the instrument and the analyzed sample. In this case, the sample was dissolved in ethanol, and UV spectra were recorded in the 200-800nm range. The absorption spectra were recorded by placing the sample solution in a quartz cuvette and measuring the absorbance at different wavelengths using a UV-visible spectrophotometer [8].

Data analysis for UV spectra involves determining the peak absorbance wavelengths and comparing them to the known spectra of standard compounds or databases of known phytochemicals. The intensity of the absorbance peak can be used to estimate the concentration of the phytochemical in the sample [9–12].

FT-IR Spectroscopy: Fourier-transform infrared spectroscopy is a powerful analytical technique for identifying and quantifying phytochemicals in plant extracts. FTIR spectroscopy measures the absorption of infrared radiation by molecules, and the resulting spectrum can provide information about the functional groups and chemical bonds present in the molecules. FTIR spectroscopy is the mathematical process of converting the measured data into a spectrum. This process transforms the time domain into a frequency domain representation, which makes it easier to analyze and interpret the data. An FTIR spectrometer equipped with ATR (Attenuated Total Reflection), Zn-Se crystal, and OPUS software (Bruker Co., Germany) was used to acquire data from the extracted phytochemicals. The ATR accessory was used to obtain the sample's spectra, allowing for

non-destructive analysis of solid, liquid, and semi-solid samples without any sample preparation. The operating conditions for FTIR analysis depend on the instrument and the sample being analyzed. In this case, the sample was placed on the ATR crystal, and the spectra were acquired over the 4000-400 cm^{-1} range [9, 11, 13–17].

Antioxidant Assays:

DPPH Radical Scavenging Assay: The DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical scavenging assay is a widely used method to determine a sample's antioxidant potential. It is based on the principle that DPPH, a stable free radical, can be reduced to a non-radical form by the hydrogen-donating ability of an antioxidant. The DPPH assay measures a compound's ability to scavenge DPPH radicals, which results in a color change from purple to yellow **Fig. 3** [18]. The absorbance value is recorded, and the percentage of scavenging activity was calculated using the following formula:

$$\% \text{ of Radical Scavenging} = (A_{control}-A_{sample}) / A_{control} \times 100$$

Where, A control is the absorbance of the control (containing only DPPH solution and methanol), and A sample is the absorbance of the test sample.

The data obtained is plotted as a percentage of radical scavenging versus the concentration of plant extract to generate a graph. The higher rate of scavenging indicates higher antioxidant activity.

The IC_{50} value (the concentration of the test compound required to scavenge 50% of DPPH radicals) can be calculated from the graph or using the Hill Langmuir equation.

$$I/I_{max} = 1 / (1+IC_{50}/A) \text{ ^n)}$$

Where 'Imax' is the maximum percent of inhibition, 'IC_{50}' is the 50% of inhibition, 'A' is the drug concentration, and 'n' is the Hill coefficient [10, 13, 19].

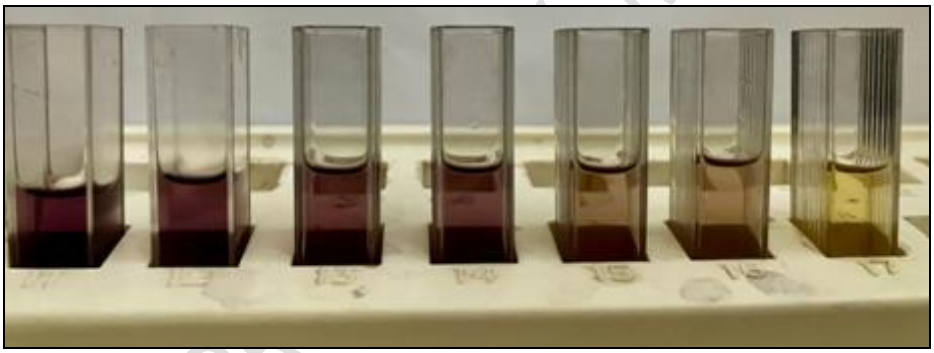

FIG. 3: DPPH RADICAL SCAVENGING RESULTS IN A COLOR CHANGE FROM PURPLE TO YELLOW

SOD Radical Scavenging Assay: Superoxide radicals are highly reactive oxygen species that can cause oxidative damage to cells and tissues. The ability of a sample to scavenge or neutralize these radicals can be measured using the superoxide radical scavenging assay. The principle of SOD activity is based on the ability of a drug to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide radicals. Riboflavin, in the presence of oxygen, generates superoxide anions and has been used based on the SOD assay. To evaluate a polyherbal formulation's superoxide radical scavenging activity, the reagents used are phosphate buffer, nitroblue tetrazolium, riboflavin, and NaCN/EDTA. The reaction mixture was prepared by adding the polyherbal formulation extract to phosphate buffer (pH=7.8), riboflavin, and nitroblue tetrazolium. NaCN/EDTA was added to the reaction mixture to stop the reaction. The absorbance of the reaction mixture was measured at 560nm using a spectrophotometer. The percent inhibition activity of SOD was calculated using the formula

$$\% \text{ of Radical Scavenging} = (A_{control}-A_{sample}) / A_{control} \times 100$$

Where the absorbance of the control is the absorbance of the reaction mixture without the sample, and the absorbance of the test sample is the absorbance of the reaction mixture with the sample [19].

Molecular Docking: It has been greatly facilitated by the dramatic growth in the availability and power of computers and the growing ease of access to small molecule and protein databases. Automated molecular docking software aims to

understand and predict molecular recognition structurally (finding likely binding modes) and energetically (predicting binding affinity). Molecular docking is usually performed between a small molecule and a target macromolecule. This is often referred to as ligand-protein docking. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization. The setting up of the input structures for the docking is just as crucial as the docking itself, and analyzing the results of stochastic search methods can sometimes be unclear. Molecular docking has a wide variety of uses and applications in drug discovery, including structure-activity studies, lead optimization, finding potential leads by virtual screening, providing binding hypotheses to facilitate predictions for mutagenesis studies, assisting x-ray crystallography in the fitting of substrates and inhibitors to electron density, chemical mechanism studies, and combinatorial library design [9, 10, 13, 16, 17, 19–22].

RESULTS AND DISCUSSIONS:

LCMS Analysis: LC/MS analysis of the prepared sample of Neuragreen was carried out with ESI ionization in both positive and negative modes. 80 molecular ions were separated and identified. From these 80 compounds, we identified nine compounds that showed high nervine activity; eight of them have in positive ionization peaks, and one has negative ionization peaks. Quadrupole mass analysis is used in this technique. The chromatogram showing positive and negative modes were depicted in **Fig. 4 & 5.**

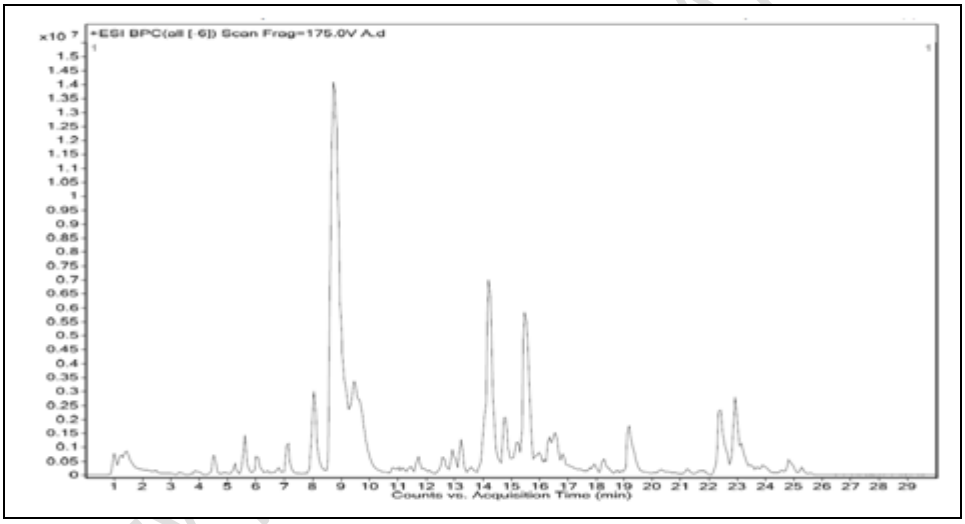

FIG. 4: CHROMATOGRAM OF THE ETHANOL EXTRACT OF THE SAMPLE NEURAGREEN AND OBTAINED IN POSITIVE MODE

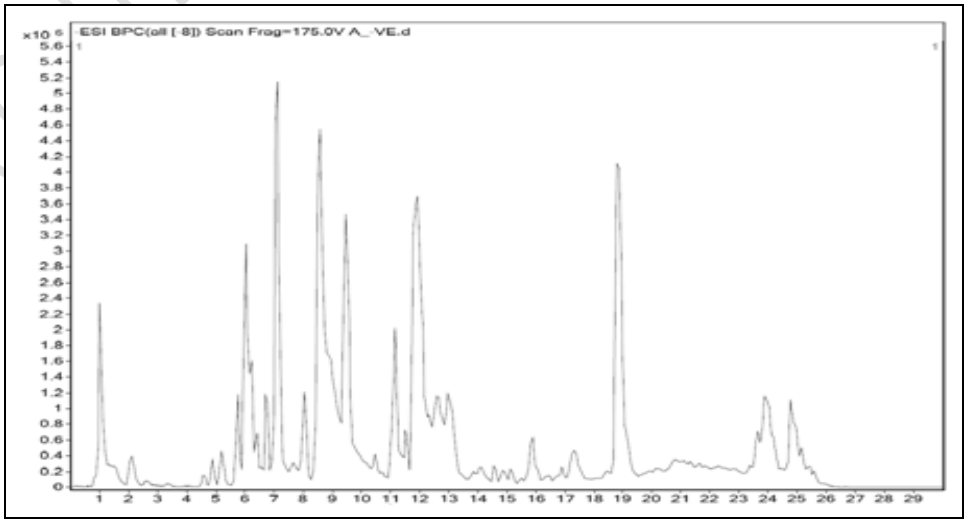

FIG. 5: CHROMATOGRAM OF THE ETHANOL EXTRACT OF THE SAMPLE NEURAGREEN AND OBTAINED IN NEGATIVE MODE

TABLE 1: COMPOUNDS FROM POSITIVE MODE OF LC/MS

Sl. no.	m/z value	Tentative identification	Molecular formula
1	141.114	Pseudotropine	$C_8H_{15}NO$
2	223.0815	Bendiocarb	$C_{11}H_{13}NO_4$
3	143.1318	Pseudoconhydrine	$C_8H_{17}NO$
4	201.1725	11-amino-undecanoic acid	$C_{11}H_{23}NO_2$
5	286.0441	Isoscutellarein	$C_{15}H_{10}O_6$
6	248.0676	Propanoic acid, 2-hydroxy-3-[(4-hydroxy-1-naphthalenyl)oxy]-	$C_{13}H_{12}O_5$
7	430.1185	N-Ethylmaleimide-S-glutathione	$C_{16}H_{22}N_4O_8S$
8	403.1814	Arg Asn Asp	$C_{14}H_{25}N_7O_7$
9	386.1549	NNAL-N-glucuronide	$C_{16}H_{24}N_3O_8$
10	302.0387	Quercetin	$C_{15}H_{10}O_7$
11	232.1548	Norfentanyl	$C_{14}H_{20}N_2O$
12	324.2159	9a-Fluoro-B-hydroxyandrosterone	$C_{19}H_{29}FO_3$
13	262.1174	Ornaline	$C_{10}H_{18}N_2O_6$
14	465.3055	Glycocholic acid	$C_{26}H_{43}NO_6$
15	459.2434	Cerivastatin	$C_{26}H_{34}FNO_5$
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-Hydroxypergolide glucuronide	$C_{25}H_{34}N_2O_7S$
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	Arg Tyr Ser	$C_{18}H_{28}N_6O_6$
21	164.1564	5-Ethyl-3-methyl-2E,4E,6E-nonatriene	$C_{12}H_{20}$
22	496.2008	Tyr Trp Glu	$C_{25}H_{28}N_4O_7$
23	495.2424	Dihydrotetrabenazine glucuronide	$C_{25}H_{37}NO_9$
24	516.2291	Perindoprilat glucuronide	$C_{23}H_{36}N_2O_{11}$
25	511.2739	AFN911	$C_{29}H_{33}N_7O_2$
26	174.1408	1,2,3,4-tetrahydro-2,5,8-trimethyl naphthalene	$C_{13}H_{18}$
27	434.2272	Pro Arg Tyr	$C_{20}H_{30}N_6O_5$
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	$C_{24}H_{34}N_8O_{12}$
33	453.2689	Delcosine	$C_{24}H_{39}NO_7$
34	604.259	6-Hydroxysandoricin	$C_{31}H_{40}O_{12}$
35	599.3039	Lividomycin B	$C_{23}H_{45}N_5O_{13}$
36	522.257	(23E)-26,26,26,27,27,27-hexafluoro-1α,25-dihydroxy-23,24-didehydrovitamin D3	$C_{27}H_{36}F_6O$
37	606.2763	Trandolapril glucuronide	$C_{30}H_{42}N_2O_{11}$
38	598.3096	PI(18:1(9Z)/0:0)	$C_{27}H_{51}O_{12}P$

TABLE 2: COMPOUNDS FROM NEGATIVE MODE OF LC/MS

Sl. no.	M/Z	Tentative identification	Molecular formula
1	332.0936	Tosyllysine Chloromethyl Ketone	$C_{14}H_{21}ClN_2O_3S$
2	342.1226	Dictyoquinazol C	$C_{18}H_{18}N_2O_5$
3	696.2013	Gnemonol A	$C_{42}H_{32}O_{10}$
4	326.1051	6-O-p-Coumaroyl-D-glucose	$C_{15}H_{18}O_8$
5	564.157	Protoaphin aglucone	$C_{30}H_{28}O_{11}$
6	424.1437	Eprosartan	$C_{23}H_{24}N_2O_4S$
7	680.2061	Copalliferol B	$C_{42}H_{32}O_9$
8	440.1388	Ginkgolide C	$C_{20}H_{24}O_{11}$
9	418.1336	2-N,6-N-Bis(2,3-dihydroxy benzoyl)-L-lysine	$C_{20}H_{22}N_2O_8$
10	430.109	Bispyribac	$C_{19}H_{18}N_4O_8$
11	564.1571	Protoaphin aglucone	$C_{30}H_{28}O_{11}$
12	534.1468	Aprepitant	$C_{23}H_{21}F_7N_4O_3$
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	$C_{20}H_{26}N_2O_2$
15	388.0869	Dopaxanthin quinone	$C_{18}H_{16}N_2O_8$

16	578.1732	eq-4"-Hydroxymaysin	$C_{27}H_{30}O_{14}$
17	432.1129	Genistein 8-C-glucoside	$C_{21}H_{20}O_{10}$
18	614.1485	CMP-N-acetylneuraminic acid	$C_{20}H_{31}N_4O_{16}P$
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	$C_{23}H_{22}ClF_3O_2$
21	788.2664	5,10-Methylenetetrahydromethanopterin	$C_{31}H_{45}N_6O_{16}P$
22	786.2496	Estradiol mustard	$C_{42}H_{50}Cl_4N_2O_4$
23	408.1496	Marmesingalactoside	$C_{20}H_{24}O_9$
24	304.1373	Nopaline	$C_{11}H_{20}N_4O_6$
25	414.1138	Asperuloside	$C_{18}H_{22}O_{11}$
26	302.0477	5,6,7,3',4'-Pentahydroxyisoflavone	$C_{15}H_{10}O7$
27	210.0363	Galactaric acid	$C_6H_{10}O_8$
28	328.2307	Auxin a	$C_{18}H_{32}O_5$
29	330.2466	9S,12S,13S-trihydroxy-10E-octadecenoic acid	$C_{18}H_{34}O_5$
30	914.5009	Tragopogonsaponin L	$C_{50}H_{74}O_{15}$
31	490.3389	7',8'-Dihydro-8'-hydroxyreticulataxanthin	$C_{33}H_{46}O_3$
32	1008.7071	Undecaprenyl phosphate mannose	$C_{61}H_{101}O_9P$
33	444.3327	N-Nitrosotomatidine	$C_{27}H_{44}N_2O_3$
34	932.4602	Scopoloside I	$C_{45}H_{72}O_{20}$
35	456.333	Citranaxanthin	$C_{33}H_{44}O$
36	488.3587	Ganoderiol D	$C_{30}H_{48}O_5$
37	440.3371	17-[[3-(1-Pyrrolidinyl)propyl]imino]androst-5-en-3beta-ol acetate	$C_{28}H_{44}N_2O_2$
38	252.16	Hexazinone	$C_{12}H_{20}N_4O_2$
39	456.3685	Oleanolic acid	$C_{30}H_{48}O_3$
40	272.24	16-Hydroxy hexadecanoic acid	$C_{16}H_{32}O_3$
41	454.3528	Ganoderal B	$C_{30}H_{46}O_3$

Among the traced seventy-nine compounds from LCMS, nine compounds exhibit high nervine activity. Three of them have positive ionization peaks, and six have negative ionization peaks.

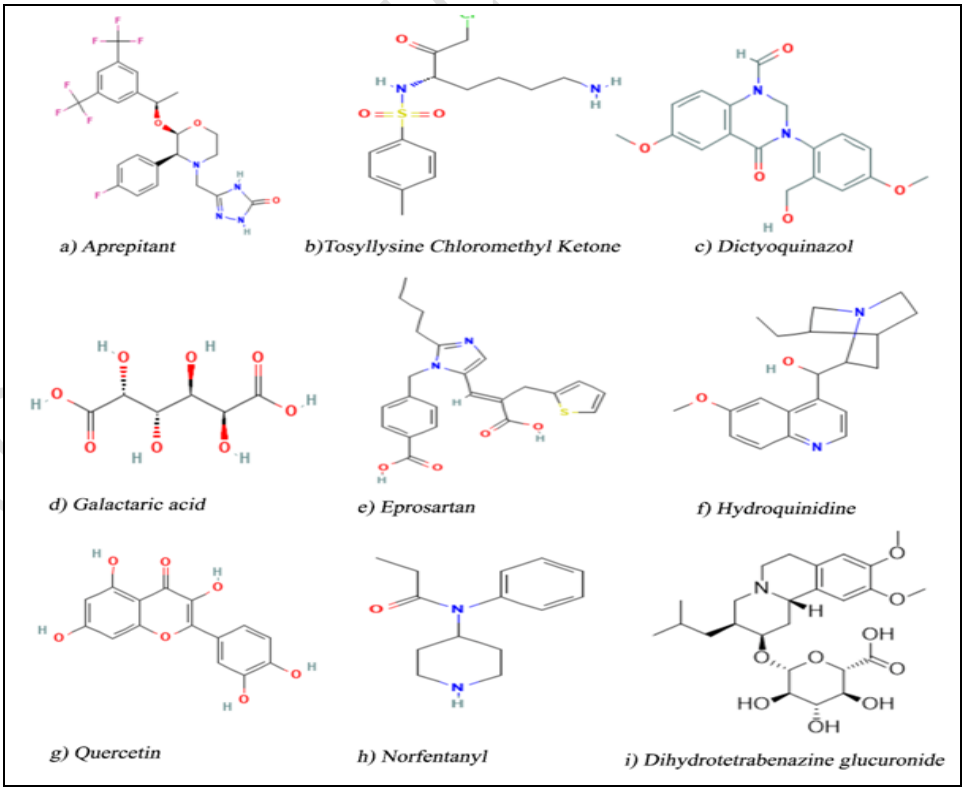

FIG. 6: CHEMICAL STRUCTURES OF VARIOUS BIOACTIVE COMPOUNDS PRESENT IN THE PREPARED POLYHERBAL FORMULATION SHOWING NERVINE ACTION.A) APREPITANT B) TOSYLLYSINE CHLOROMETHYL KETONE C) DICTYOQUINAZOL D) GALACTARIC ACID E) EPROSARTAN F) HYDROQUINIDINE G) QUERCETIN H) NORFENTANYLI) DIHYDROTETRABENAZINE GLUCURONIDE

They are tosyllysine chloromethyl ketone, eprosartan, aprepitant, galactaricacid, norfentanyl, cis-dihydrotetrabenazine glucuronide, quercetin, hydroquinidine, dictyoquinazol c, which are the nine compounds showing nervine action. The m/z 332.0936- tosyl lysine chloromethyl ketone, 424.1437- eprosartan, 534.1468 - aprepitant, 210.0363 - galactaricacid, 232.1548- nor fentanyl, 495.2424 – cis-dihydro tetrabenazine glucuronide, 302.0387 - quercetin and 326.1971- hydroquinone and 342.1226- dictyoquinazol c. All these compounds have neuroprotective action. Besides, these compounds show biological properties such as antioxidant and anti-inflammatory properties. The structure of these nine compounds is given in **Fig. 6.**

Spectroscopic Analysis:

UV- VIS Spectroscopy: Since, spectra can be considered as a fingerprint of each authenticated standard formulation, UV-Vis spectroscopic analysis can be used as a quality control in Ayurvedic formulations. Based on the UV-VIS spectroscopy analysis, it appears that the ethanolic extract of the polyherbal formulation contains compounds with C=O and C=C functional groups. These functional groups are typically found in various classes of phytochemicals, including flavonoids, terpenes, and alkaloids, among others. It is based on the electronic absorption caused by the compounds present in plants and those formed during manufacturing chemical reactions. Electronic transitions of molecules result in the absorption of UV or visible light. The absorption of UV-Vis radiation by a molecule can be explained by the excitation of electrons from their ground state to higher energy states.

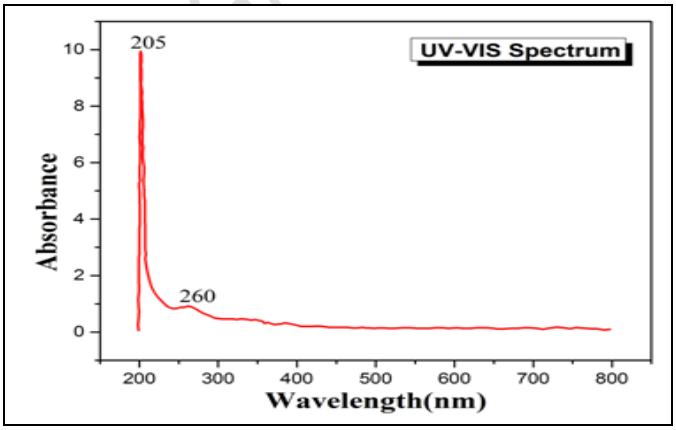

FIG. 7: CALCULATED UV-VISIBLE SPECTRUM OF NEURAGREEN POLYHERBAL FORMULATION

The electronic absorptions observed in the ethanolic extract of the polyherbal formulation at 205 nm and 260 nm correspond to the electronic transitions associated with the C=O and C=C functional groups respectively **Fig. 7.** At 205 nm, the sharp peak observed in the UV-Vis spectrum indicates that the electronic transition corresponds to the excitation of electrons from the non-bonding π-electron lone pairs of the oxygen atom to the antibonding π* orbitals of the carbonyl group. This transition is known as the π→π∗ transition and is characteristic of compounds containing a carbonyl group, such as flavonoids, coumarins, or quinones. The sharp peak observed at 205 nm indicates the presence of a C=O functional group, which could be attributed to the presence of flavonoids, such as flavones or flavonols, or other compounds such as coumarins, quinones or steroids. These compounds have been reported to have a range of biological activities, including antioxidant, anti-inflammatory, and neuroprotective properties. At 260 nm, the broad peak observed in the UV-VIS spectrum indicates that the electronic transition corresponds to the excitation of π-electrons from the double bond of the C=C functional group to the π* antibonding orbitals. This transition is known as the π→π∗ transition and is characteristic of compounds containing double bonds, such as terpenes, stilbenes, or lignans. The broad spectrum observed at 260 nm indicates the presence of C=C functional groups, which could be attributed to the presence of terpenes or other compounds such as lignans or stilbenes. Terpenes are known to possess various biological activities, including anxiolytic, sedative, and analgesic properties, which could be beneficial for promoting nervous system health. It is important to note that the absorption peak observed in UV-Vis spectroscopy is affected by various factors, such as solvent polarity, pH, and molecular environment. Therefore, the electronic transitions observed in the UV-Vis spectrum can vary depending on the specific functional groups and compounds present in the polyherbal formulation.

FTIR Spectroscopy: The FTIR spectrum of the polyherbal formulation **Fig. 8** revealed the presence of various functional groups, which indicates the presence of various phytochemicals that have been reported to possess various potential therapeutic applications.

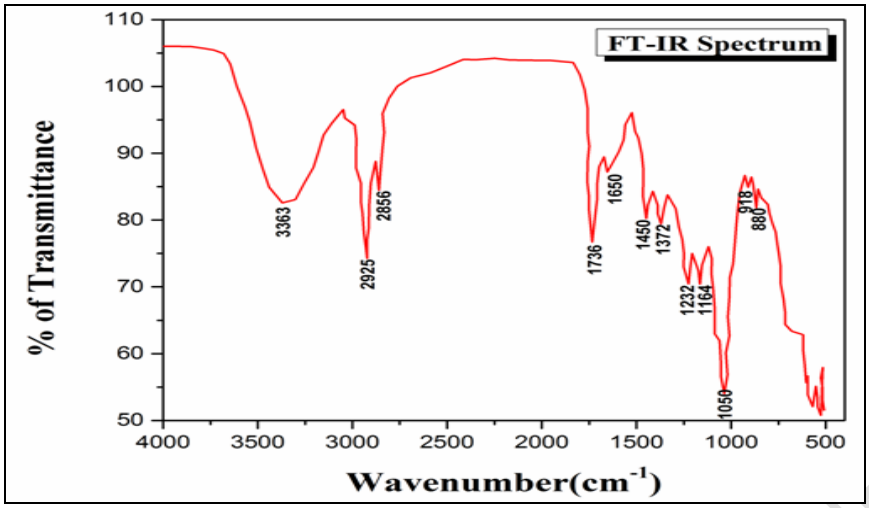

FIG. 8: FTIR SPECTRUM REPRESENTING POTENTIAL BANDS IN THE ETHANOLIC EXTRACT OF THE FORMULATION

Based on the FTIR spectrum, the polyherbal formulation contains various functional groups such as quinone oximes, cellulose, C=C conjugated with C=C C=O, R-CH_3-$C(CH_3)_2$, -$OC(CH_3)_2$, and P-CH_3. The peak at 3353 cm^{-1} is attributed to broad OH stretching vibration (associated with quinone oximes), while the peaks at 2925 cm^{-1} and 2855 cm^{-1} correspond to C-H stretching vibration of acyclic -CH_2- group. The peak at 1736cm^{-1} represents the C=O stretching vibration of oxidized cellulose. The peak at 1736cm^{-1} represents the C=O stretching vibration of oxidized cellulose. The peak at 1650 cm^{-1} indicates the presence of C=C conjugated with C=C C=O. The peaks at 1456 cm^{-1} and 1372 cm^{-1} correspond to the asymmetric and symmetric C-H deformation vibration of R-CH_3 group, respectively. The peak at 1232 cm^{-1} is attributed to the C-H deformation vibration of cyclobutene, while the peak at 1164cm^{-1} corresponds to the C-C stretching vibration of -$C(CH_3)_2$ group. The peak at 1050 cm^{-1} represents the C-O stretching vibration of primary alcohols, specifically R-CH_2-CH_2-OH which may be the presence of ethanolic extract in the polyherbal formulation. The peaks at 918cm^{-1} and 880cm^{-1} correspond to the C-C skeleton vibration of cyclobutane and C-H rocking vibration of P-CH_3 group, respectively. Overall, the FTIR spectrum suggests that the polyherbal formulation contains a complex mixture of compounds with various functional groups. The presence of quinone oximes, cellulose, and conjugated C=C, C=O suggests the presence of polyphenolic compounds with potential antioxidant activity. The presence of cyclobutane suggests the presence of terpenoids.

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

Peak number	Band Range (literature) Wavenumber (cm^{-1})	Band Range (experimental) Wave number (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	Alkene (C=C),	Carotenoids,

				conjugate with C=C C=O	carbonyl (C=O)	flavonoids
6	1440-1465	1456	C-H deformation (assymetric)	R-CH_3	Methyl (-CH_3)	Fatty acids, esters, aldehydes
7	1390-1370	1372	C-H deformation (symmetric)	R-CH_3	Methyl (-CH_3)	Fatty acids, 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

Antioxidant Assays:

DPPH Assay: The DPPH radical scavenging assay is a widely used method to determine the antioxidant activity of natural products. The assay is based on the principle of electron transfer, where the DPPH radical, a stable free radical, accepts an electron or hydrogen radical from an antioxidant molecule to form a stable diamagnetic molecule. This reaction leads to a decrease in the absorbance of DPPH at 517nm, which can be measured using a spectrophotometer. In this assay, the DPPH radical is reduced by an antioxidant molecule, resulting in a decrease in absorbance at 517nm. The extent of reduction of DPPH radicals is directly proportional to the antioxidant activity of the test sample. In the present study, the antioxidant activity of a polyherbal formulation as a nervine tonic was evaluated using the DPPH radical scavenging assay. The control and all the test samples are incubated in the dark for 15-20 minutes, and the absorbance is measured at 517nm using a microplate reader. The data obtained is plotted as a percentage of radical scavenging versus the concentration of plant extract to generate a graph. The graph **Fig. 9** shows an increasing trend, indicating that the percentage of radical scavenging of DPPH radical increased with increasing concentrations of the polyherbal formulation. This suggests that the polyherbal formulation contains a high concentration of antioxidant molecules that can effectively scavenge the DPPH radical. The Hill Langmuir Equation was used to calculate the IC_{50} value, which represents the concentration of the plant extract required to scavenge 50% of the DPPH radical. The IC_{50} value of the polyherbal formulation was found to be 7.3µg/ml, indicating strong antioxidant activity of the formulation. The decrease in absorbance at 517nm indicates that DPPH radical by the polyherbal formulation, leading to the formation of a stable diamagnetic molecule and resulting in a colorless methanol solution **Fig. 9**.

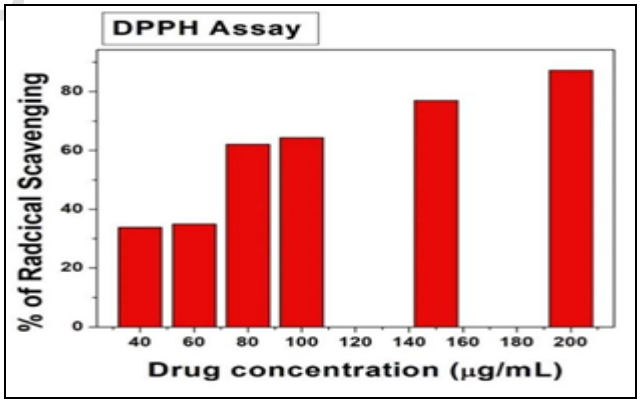

FIG. 9: DPPH RADICAL SCAVENGING ACTIVITY OF POLYHERBAL FORMULATION AT VARYING CONCENTRATIONS. The percentage of radical scavenging increases with drug concentration, indicating dose-dependent antioxidant activity. The highest radical scavenging (around 80%) is observed at 200 µg/ml, while the lowest (around 30%) occurs at 40 µg/ml.

SOD Assay: Superoxide scavenging activity is an important antioxidant activity that can be evaluated by determining the nitroblue tetrazolium (NBT) reduction method by McCord and Fridovich, 1969. For this study, the reaction mixture was prepared by adding the polyherbal formulation extract to phosphate buffer, riboflavin, and nitroblue tetrazolium. NaCN/EDTA was added to the

reaction mixture to stop the reaction. The absorbance of the reaction mixture was measured at 560nm.

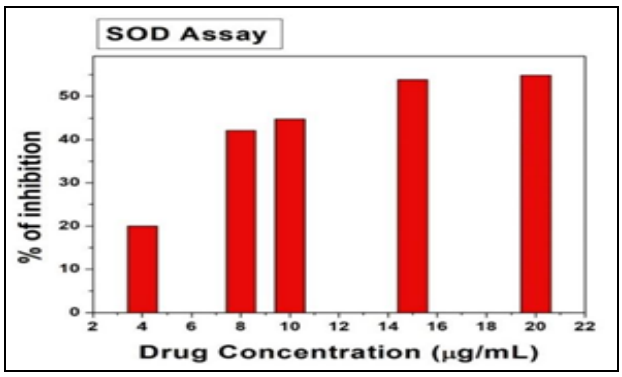

FIG. 10: SOD RADICAL SCAVENGING ACTIVITY OF POLYHERBAL FORMULATION AT VARYING CONCENTRATIONS. The percentage of radical scavenging increases with drug concentration, indicating dose-dependent antioxidant activity

The results of the assay were plotted as a graph of the percentage of radical scavenging versus the concentration of the formulation. The IC_{50} value was calculated using the Hill Langmuir equation and was found to be 14.9 µg/ml. A higher percentage of inhibition activity indicates a higher SOD activity and thus, a higher antioxidant capacity. The IC_{50} value obtained in this study suggests that the polyherbal formulation has significant SOD radical scavenging activity and, thus, a potent antioxidant capacity. The results of this study are consistent with previous studies that have shown that the polyherbal formulation has significant antioxidant activity. The phytochemicals present in the polyherbal formulation, such as flavonoids and phenolic compounds, have been reported to possess antioxidant activity. The antioxidant activity of the polyherbal formulation may be attributed to the synergetic effect of these phytochemicals. The findings of this study suggest that the polyherbal formulation may be used as a natural source of antioxidants and as a potential therapeutic agent for the prevention and treatment of oxidative stress-related disorders.

Docking Studies:

Docking with MAO – B: Nervine tonics are often used to promote relaxation, reduce anxiety, and improve mental clarity. These effects can be attributed to the modulation of neurotransmitters in the brain. MAO - B (monoamine oxidase B) is an enzyme involved in the breakdown of certain neurotransmitters, such as dopamine and serotonin. By inhibiting the activity of MAO - B, the breakdown of dopamine is slowed down, leading to increased dopamine levels in the brain. Additionally, MAO - B inhibitors have been found to have neuroprotective effects, meaning they can help protect nerve cells from damage or degeneration. This is particularly relevant in the context of neurological disorders such as Parkinson's disease, where MAO - B inhibitors are commonly used as a therapeutic approach to delay the progression of the disease. In the context of nervine tonics, which are substances that support and maintain the health and functioning of the nervous system, the role of MAO - B is mainly centered around maintaining an appropriate balance of neurotransmitters. Hence, MAO - B helps regulate the levels of dopamine, a neurotransmitter associated with motivation, reward, and pleasure. This can contribute to improved mood and feelings of well-being [23]. The action mechanism is given in the **Fig. 11A.**

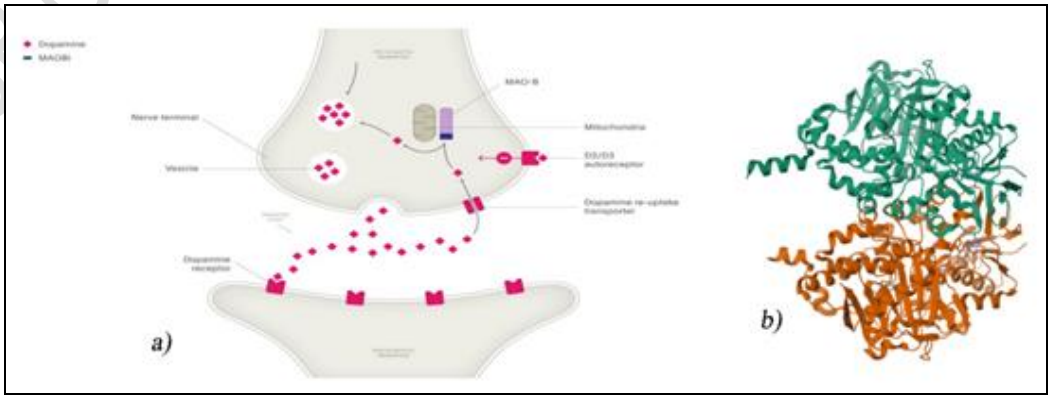

FIG. 11: THE MECHANISM OF MAO - B (REF) B) STRUCTURE OF MONOAMINE OXIDASE B ENZYME TAKEN FROM PROTEIN DATA BANK

2V5Z is a 2-chain structure with sequences from Homo sapiens is used in docking process. **Fig. 11B** represents the structure of monoamine oxidase B enzyme taken from protein data bank.

The selected nine compounds as shown in **Fig. 11** are docked with MAO - B protein. **Fig. 12** demonstrates the 3-dimensional protein-ligand interaction and **Fig. 13** demonstrates the 2-dimensional interaction of all selected compounds in the dynamic site of MAO-B obtained from the graphical interface Maestro. All the selected active ligands are found to be buried in the deep binding pocket of MAO-B in an indistinguishable way. All the ligands interact with the active site's amino acids of the protein by H-bonding and π bonds, which is depicted in red and dotted lines.

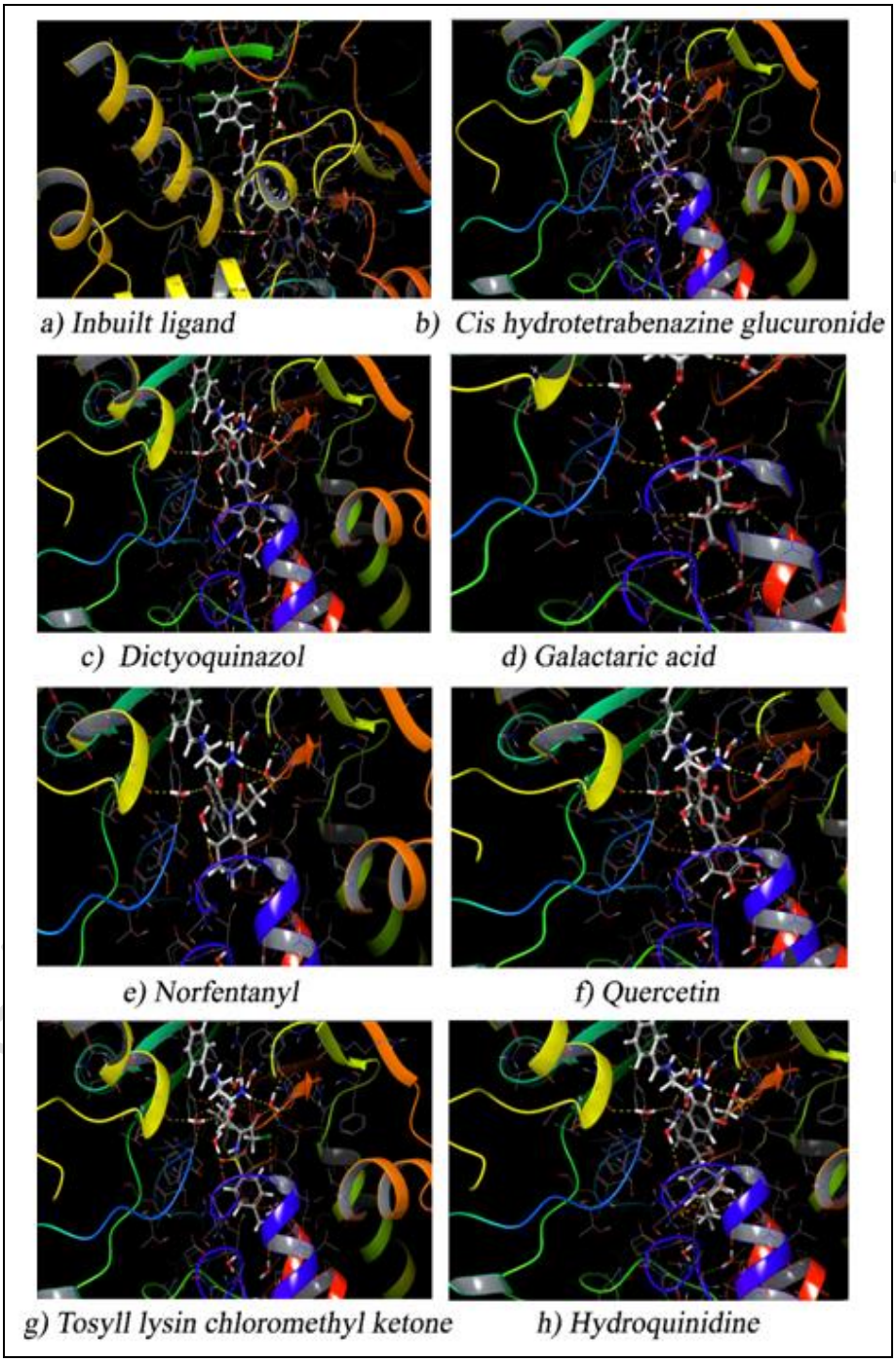

FIG. 12: 3D INTERACTION PICTURE OF SCREENED BIO ACTIVE COMPOUNDS IN POLYHERBAL FORMULATION NEURAGREEN WITH MAO - B TARGET PROTEIN (A) INBUILT LIGAND, (B) CIS HYDROTETRABENAZINE GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC ACID, (E) NORFENTANYL, (F) QUERCETIN, (G) TOSYLL LYSIN CHLOROMETHYL KETONE,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

Fig. 13 depicts the 2D interaction picture of the docking analysis of screend bioactive compounds ligands with Monoamine Oxidase B (MAO-B), several key interactions with amino acid residues within the active site were identified, providing insights into the binding mechanisms and potential inhibitory effects of the compounds.

The inbuilt ligand, serving as a reference, displayed hydrophobic interactions with ILE199 and TYR326, along with π-π stacking interactions involving TYR60 and TYR398, which are crucial for stabilizing the ligand within the binding pocket. This interaction pattern sets the baseline for comparison with the other ligands. Cis hydrotetrabenazine glucuronide exhibited strong hydrogen bonding with SER59 and GLY434, in addition to hydrophobic interactions with TYR398 and MET60, suggesting a well-fitted placement within the MAO-B active site. Similarly, dictyoquinazol showed prominent hydrophobic interactions with LEU171 and TYR398, alongside π-π interactions with TYR60, highlighting its ability to form stable binding through non-polar interactions. Galactaric acid, with its multiple hydroxyl groups, formed extensive hydrogen bonds with GLY434, SER59, and ARG58, indicating strong polar interactions that could enhance its inhibitory potential, supplemented by π-π stacking with TYR398 and TYR60.

Norfentanyl's interaction profile was dominated by hydrophobic interactions with ILE199, TYR398, and LEU171, while also engaging in hydrogen bonding with SER59 and GLY434, reinforcing its potential for stable binding within MAO-B. Quercetin, a well-known flavonoid, formed hydrogen bonds with SER59, GLY434, and ARG58, with additional π-π stacking involving TYR398 and TYR60, underscoring its effective interaction through both polar and non-polar mechanisms. Tosyll lysin chloromethyl ketone, a reactive molecule, likely formed covalent bonds with SER59, in addition to engaging in hydrophobic and hydrogen bond interactions with LEU171 and GLY434, indicating a strong inhibitory potential through covalent modification. Lastly, hydroquinidine demonstrated a balanced interaction pattern, forming hydrogen bonds with SER59 and GLY434, and π-π stacking with TYR398 and TYR60, similar to the other aromatic compounds. The combination of hydrophobic interactions and hydrogen bonds across all ligands suggests a multi-faceted approach to inhibition, where compounds leverage both polar and non-polar residues to achieve optimal binding.

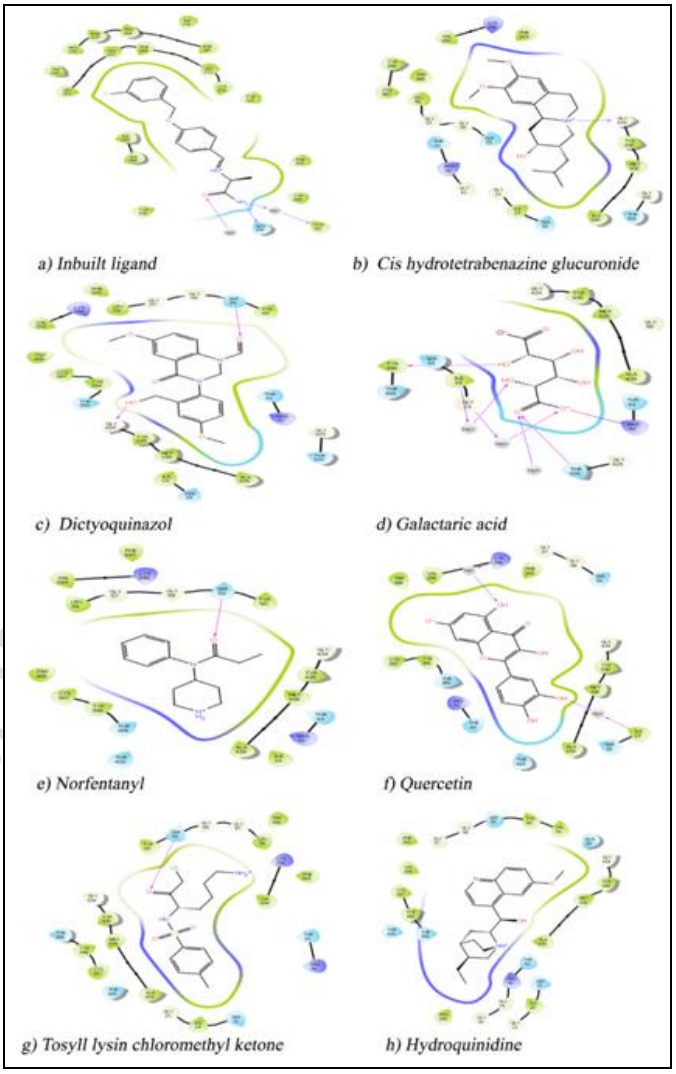

FIG. 13: 2D INTERACTION DIAGRAMS OF MOLECULAR DOCKING STUDIES INVOLVING THE SCREENED BIOACTIVE COMPOUNDS FROM THE POLYHERBAL FORMULATION NEURAGREEN WITH NERVINE ACTION AND THE ENZYME MONOAMINE OXIDASE B (MAO-B). (A) INBUILT LIGAND, (B) CIS HYDROTETRA-BENAZINE-GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC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

The docking scores and binding energies were tabulated in **Table 4** that provide valuable insights into the efficacy of the selected bioactive compounds. The inbuilt ligand (2V5Z) exhibited the strongest interaction with MAO-B, having a docking score of -21.250 and a binding energy of -

350.664 kcal/mol, serving as a reference for comparison. Among the tested compounds, quercetin demonstrated the highest affinity, with a docking score of -9.542 and a binding energy of -84.403 kcal/mol, suggesting strong binding stability. Dictyoquinazol followed closely, with a docking score of -9.385 and a binding energy of -51.615 kcal/mol, indicating its potential for effective inhibition. Tosyll lysin chloromethyl ketone and norfentanyl also showed moderate binding affinities, with docking scores of -7.469 and -7.268, respectively. Despite lower docking scores, galactaric acid, cis-dihydrotetrabenazine glucuronide, and hydroquinidine still displayed binding energy values that suggest moderate interactions, though not as robust as the inbuilt ligand or quercetin. The relatively higher docking scores and binding energies of quercetin and dictyoquinazol highlight their potential to be developed as nervine tonics due to their ability to inhibit MAO-B, increasing neurotransmitter availability and offering neuroprotective benefits.

These compounds' ability to interact effectively with key residues of MAO-B highlights their potential as candidates for nervine tonics. By inhibiting MAO-B, these ligands could increase the levels of dopamine and other monoamines in the brain, which would help to stabilize mood, improve cognitive function, and provide neuroprotection. Additionally, many of these compounds, such as quercetin, also possess antioxidant properties, which further support their role in reducing oxidative stress, a key factor in neurodegenerative diseases.

TABLE 4: DOCKING SCORES AND BINDING ENERGY OF THE LIGANDS WITH MAO - B PROTEIN

Sample	Docking Score	Binding Energy Kcal/mol
Inbuilt ligand 2V5Z	-21.250	-350.664
Quercetin	-9.542	-84.403
Dictyoquinazol	-9.385	-51.615
Tosyllysin chloromethyl ketone	-7.469	-60.221
norfentanyl	-7.268	-51.462
Cis-dihydrotetrabenazine glucuronide	-7.030	-46.778
hydroquinidine	-6.622	-38.369
Galactaric acid	-6.416	-65.294

MAO-B with inbuilt ligand coordinates N from NH_2 and TYR 60 through a H_2O molecule and the same N coordinated to GLN 206. It has a docking score of -21.250 and a binding energy -350.664 kcal/mol. Cis hydrotetrabenazine glucuronide ligand coordinates with GLY 434 through N of the NH+ group having a docking score of -7.030 and binding energy -46.778 Kcal/mol.

Dictyoquinqzol coordinates at 2 sites carbonyl oxygen to SER 59 and OH to GLY 434 having a docking score of -9.385 and binding energy of -51.615 Kcal/mol. Galactaric acid coordinates at three sites through H_2O. O^- to ARG 42, carbonyl oxygen to THR 426. OH to TYR 98, ILE and OH coordinate through H_2O. One H_2O is directly coordinate to carbonyl oxygen, it has a docking score of -6.416 and binding energy of -65.294 K cal/mol. Norfentanyl ligand coordinates SER 59 to carbonyl oxygen; it has a docking score of -7.030 and binding energy -51.465 Kcal/mol. Quercetin ligands have two coordination sites through H_2O. LYS 296 to OH through H_2O. ILE 14 to another OH through H2O.Docking score is -9.542 and binding energy -84.403 k cal/mol. Tosyllysin chloromethyl ketone ligand has one coordination of SER 59 to carbonyl oxygen. The docking score is -7.469 and the binding energy -60.221Kcal/mol. Hydroquinidine ligand there is an electrostatic force that coordinates ligand to the protein. It has docking score -6.622 and binding energy of -38.369 kcal/mol. The high docking score is for quercetin -9.542.

Docking with GABA: Gamma-aminobutyric acid (GABA) is an amino acid that serves as the primary inhibitory neurotransmitter in the brain and a major inhibitory neurotransmitter in the spinal cord. GABA is a neurotransmitter that plays a crucial role in the nervous system. In the context of nervine tonics, GABA is often involved in promoting relaxation, reducing anxiety, helping to calm and balance brain activity, and improving sleep quality. Many nervine tonics aim to increase GABA levels or enhance the effects of GABA in the brain and also bind to GABA receptors on

nerve cells, leading to a decrease in neuronal excitability and a reduction in the firing of nerve impulses. They may inhibit enzymes that degrade GABA, increasing its levels and prolonging its effects. Furthermore, these tonics may interact with GABA receptors, enhancing the binding of GABA. This can result in a sense of calmness and relaxation. The clinical significance of GABA cannot be underestimated. Disorders in GABA signaling are implicated in a multitude of neurologic and psychiatric conditions. Modulation of GABA signaling is the basis of many pharmacologic treatments in neurology, psychiatry, and anesthesia [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. Dysfunctional GABA interneuron activity can disrupt the excitatory/inhibitory (E/I) balance in the cortex, which could represent a core pathophysiological mechanism underlying cognitive dysfunction in schizophrenia. Recent research suggests that selective modulation of the GABA-ergic system is a promising intervention for the treatment of schizophrenia-associated cognitive defects [25]. The action mechanism is given in the **Fig. 14.**

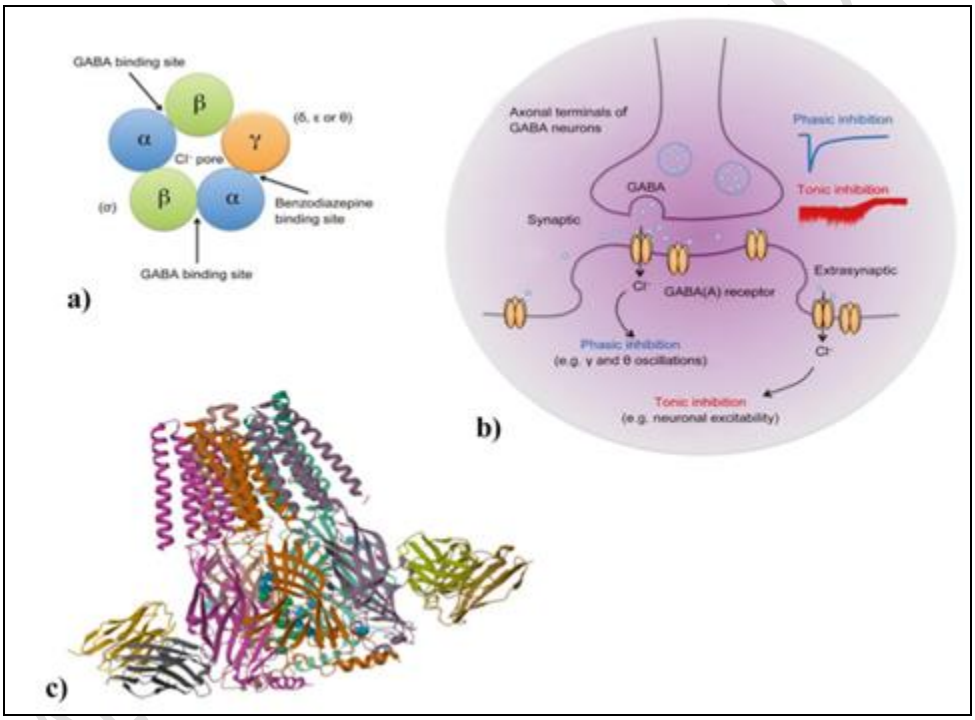

FIG. 14: MECHANISM OF GABA (REF) C) STRUCTURE OF GAMMA-AMINOBUTYRIC ACID ENZYME TAKEN FROM PROTEIN DATA BANK

Fig. 15 demonstrates the 3-dimensional protein-ligand interaction and **Fig. 16** demonstrates the 2-dimensional interaction of all selected compounds in the dynamic site of the GABA protein obtained from the graphical interface Maestro. All the selected active ligands are found to be buried in the deep binding pocket of GABA in an indistinguishable way. All the ligands interact with the active site amino acids of the protein by H-bonding and π bonds, which are depicted in red and dotted lines.

The interactions observed between the screened bioactive compounds in the polyherbal formulations and the GABA receptor were depicted in **Fig. 16**, that provide important insights into their potential as modulators of GABA neuro-transmission, balance between excitatory and inhibitory signals in the nervous system. GABA receptors are a major target for drugs that treat conditions such as anxiety, insomnia, and epilepsy, making these interactions highly relevant for exploring potential therapeutic benefits.

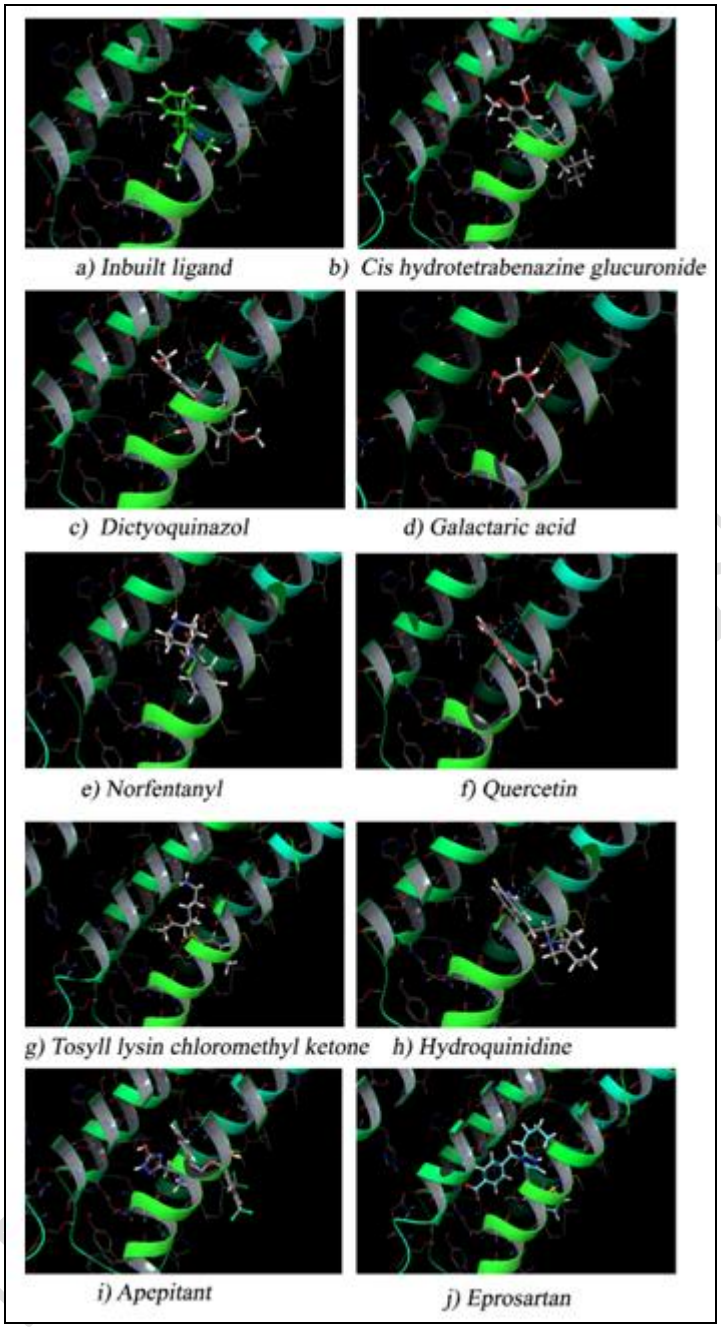

FIG. 15: 3D INTERACTION PICTURE OF SCREENED BIO ACTIVE COMPOUNDS IN POLYHERBAL FORMULATION NEURAGREEN WITH GABA TARGET PROTEIN (A) INBUILT LIGAND, (B) CIS HYDROTETRABENAZINE GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC ACID, (E) NORFENTANYL, (F) QUERCETIN, (G) TOSYLL LYSIN CHLOROMETHYL KETONE, (H) HYDROQUINIDINE, (I) APEPITANT AND J)EPROSARTAN

The inbuilt ligand shows strong hydrophobic interactions with key residues like LEU259, ASN265, PHE289, and MET236. These residues are often involved in stabilizing ligands in the binding pocket of GABA receptors through nonpolar interactions, particularly with aromatic rings, possibly involving π-π stacking and hydrophobic interactions. Such strong binding suggests a significant inhibitory effect on the receptor, which could contribute to modulating inhibitory signaling in the brain. Cis hydrotetrabenazine glucuronide exhibits both hydrophobic interactions and hydrogen bonding with residues such as ASN265, THR267, and PHE289. The presence of fluorine in the ligand structure may contribute to additional van der Waals forces or halogen bonds, enhancing its binding affinity. These interactions suggest that cis hydrotetrabenazine glucuronide may stabilize the receptor in a way that could potentially enhance

GABAergic activity, making it a promising candidate for modulating the receptor's inhibitory effects. Dictyoquinazol forms strong hydrophobic interactions with LEU259, PRO255, and ASN265, with the potential for hydrogen bonding through ASN265. These interactions highlight the ligand's ability to bind effectively within the GABA receptor's active site, potentially modulating its function. The hydrophobic stabilization within the receptor may indicate that dictyoquinazol could influence the receptor's inhibitory signaling pathways, which is a desirable trait for compounds aimed at calming the nervous system. Galactaric acid forms multiple hydrogen bonds with residues such as ASN265 and THR267, indicative of strong polar interactions.

The presence of several hydroxyl groups in galactaric acid likely contributes to these hydrogen bonds, further stabilizing the ligand in the receptor's binding pocket. This could point to its potential role in modulating GABA receptor activity, possibly leading to enhanced inhibitory neurotransmission, which is beneficial in reducing excitatory imbalances that lead to anxiety or seizure activity. For norfentanyl, interactions with residues like ASP230 and LEU259 suggest the formation of salt bridges or ionic interactions, alongside hydrophobic interactions with LEU259, providing stable binding within the active site. These interactions could contribute to an effective modulation of the receptor, where the ligand might influence the receptor's ability to mediate inhibitory signaling, making it a potential candidate for nervous system-related applications.

Quercetin shows several important hydrogen bonds with THR267, ASN265, and LEU236, along with hydrophobic interactions with LEU259 and PHE289. Given quercetin's polyphenolic structure, it is likely that these interactions involve both π-π stacking and hydrogen bonding, leading to a stable ligand-receptor complex. Quercetin's well-established antioxidant and anti-inflammatory properties, combined with its potential modulation of the GABA receptor, suggest that it could play a dual role in neuroprotection and mood regulation. Tosyll lysin chloromethyl ketone shows potential covalent bond formation with THR267, along with hydrophobic interactions with LEU259 and MET236. These interactions point to a possible irreversible inhibition of the receptor, which could lead to sustained modulation of GABAergic signaling. This type of interaction is especially relevant for drugs that require prolonged receptor inhibition, such as those targeting chronic anxiety or seizure disorders. Hydroquinidine interacts through hydrogen bonding with ASP230 and shows hydrophobic interactions with LEU259 and PHE289, suggesting a stable binding configuration. The balanced combination of polar and nonpolar interactions implies that hydroquinidine could be an effective modulator of the GABA receptor, potentially enhancing inhibitory neurotransmission and promoting a calming effect on the nervous system.

Aprepitant forms hydrogen bonds with ASN265 and ARG254, in addition to hydrophobic interactions with residues like LEU259, MET236, and PHE289. This interaction profile indicates that aprepitant could effectively stabilize the GABA receptor, enhancing its inhibitory action and providing therapeutic potential for conditions involving excessive neuronal excitability, such as epilepsy or anxiety disorders. Lastly, Eprosartan shows interactions with LEU259 and ARG234, suggesting the formation of ionic interactions, along with hydrophobic contacts with ILE262. These interactions likely stabilize the ligand within the receptor's binding pocket, contributing to effective modulation of GABAergic neurotransmission. Eprosartan's ability to modulate the receptor could provide neuroprotective or calming effects, making it a potential candidate 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 score	Binding Energy kCal/Mol
Inbuilt ligand 6x3x	-9.003	-67.749
Dictyoquinazol	-7.904	-54.191
Quercetin	-7.398	-50.023
Eposartan	-6.909	-47.572
Tosyllysin chloromethyl ketone	-6.851	-63.102
Norfentanyl	-6.807	-47.432
Hydroquinidine	-6.784	-35.336
Cisdihydrotetrabenzine glucuronide	-6.618	-29.040
Apepitant	-7.051	-39.725
Galactaric acid	-3.921	-27.454

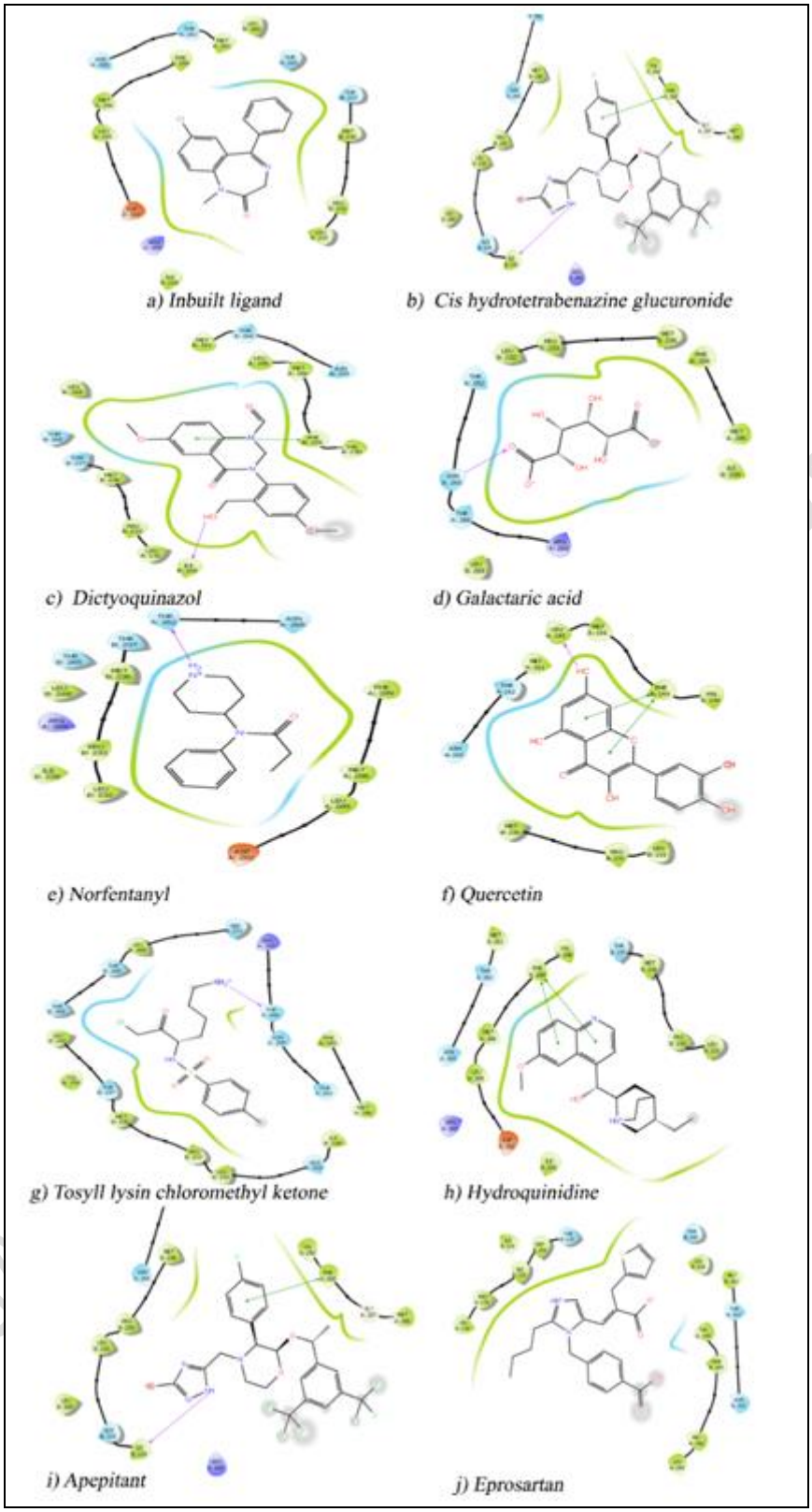

FIG. 16: 2D INTERACTION PICTURE OF SCREENED BIO ACTIVE COMPOUNDS IN POLYHERBAL FORMULATION NEURAGREEN WITH GABA TARGET PROTEIN (A) INBUILT LIGAND, (B) CIS HYDROTETRABENAZINE GLUCURONIDE, (C) DICTYOQUINAZOL, (D) GALACTARIC ACID, (E) NORFENTANYL, (F) QUERCETIN, (G) TOSYLL LYSIN CHLOROMETHYL KETONE, (H)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

The docking scores and binding energies for the screened ligands interacting with the GABA receptor were tabulated in **Table 5.** The inbuilt ligand, with the most negative docking score of -

9.003 and a binding energy of -67.749 kcal/mol, exhibits the strongest interaction, indicating a high binding affinity and stability in the receptor's binding pocket. This sets the standard for comparison. Dictyoquinazol, with a docking score of -7.904 and a binding energy of -54.191 kcal/mol, also shows strong binding, indicating that it may effectively modulate the receptor's activity. Similarly, quercetin, with a docking score of -7.398 and binding energy of -50.023 kcal/mol, demonstrates a good binding profile, suggesting it could interact well with the receptor, possibly due to its polyphenolic structure and ability to form hydrogen bonds. Eprosartan and tosyllysin chloromethyl ketone, with docking scores of -6.909 and -6.851 respectively, show moderate binding, but tosyllysin chloromethyl ketone's high binding energy of -63.102 kcal/mol suggests stronger and more stable interactions, likely due to covalent or irreversible binding. Norfentanyl also demonstrates moderate binding with a docking score of -6.807 and binding energy of -47.432 kcal/mol, indicating it could still be effective in modulating the receptor, though not as potent as the top ligands. Hydroquinidine and cis dihydrotetrabenazine glucuronide, with docking scores of -6.784 and -6.618 respectively, show weaker binding, with lower binding energies indicating less favorable and less stable interactions with the receptor. Aprepitant, with a docking score of -7.051 and binding energy of -39.725 kcal/mol, demonstrates moderate binding but shows less stability compared to some other ligands. Galactaric acid, with the lowest docking score of -3.921 and binding energy of -27.454 kcal/mol, shows the weakest interaction, suggesting limited potential as a GABA receptor modulator.

The docking study results indicate how different compounds in the polyherbal formulation could interact with the GABA receptor, which plays a crucial role in regulating neuronal excitability through the mediation of inhibitory neurotransmission. This is especially relevant for nervine tonics, which are intended to calm and restore balance to the nervous system.

CONCLUSION: This study provides a detailed investigation into the novel polyherbal nervine tonic formulation, Neuragreen, through a combination of advanced analytical techniques and molecular docking studies. The LC-MS, UV-Visible, and FTIR spectroscopic analyses confirmed the presence of key bioactive compounds, including flavonoids, terpenoids, saponins, and alkaloids, which are known to possess antioxidant and neuroprotective properties. The antioxidant assays (DPPH and SOD) demonstrated strong antioxidant capacity, further supporting the therapeutic potential of the formulation against oxidative stress-related disorders.

The FTIR spectrum suggests that the polyherbal formulation contains a complex mixture of compounds with various functional groups. The presence of quinone oximes, cellulose, and conjugated C=C C=O suggests the presence of polyphenolic compounds with potential antioxidant activity. The presence of cyclobutane and the $C(CH_3)_2$ group suggests the presence of terpenoids. In DPPH radical scavenging activity, the IC_{50} value of the polyherbal formulation was found to be 7.3µg/mL, indicating a strong antioxidant activity of the formulation, whereas in SOD enzyme activity, the IC_{50} value obtained in this study was found to be 14.9µg/mL, which suggests that the polyherbal formulation has significant SOD radical scavenging activity and thus a potent antioxidant capacity.

From LCMS chromatogram analysis, nine phytochemicals responsible for nervine activity were screened; Cis hydrotetrabenazine glucuronide, Dictyoquinazol, Galactaric acid, Norfentanyl, Quercetin, Tosyll lysine chloromethyl ketone, Hydroquinidine, Apepitant and Eprosartan, and their complex mechanisms were studied using molecular docking. The docking studies with both the MAO-B and GABA proteins reveal significant insights into the potential activity of the polyherbal formulation. For MAO-B, several compounds, including dictyoquinazol, quercetin, and tosyllysin chloromethyl ketone, exhibited strong binding affinities, indicating their potential to inhibit the enzyme's activity. Inhibition of MAO-B could lead to increased levels of neurotransmitters like dopamine, which is critical in managing neurological disorders, enhancing mood, and supporting cognitive function. On the other hand, the docking studies with GABA receptor suggest that compounds like dictyoquinazol, quercetin, and

tosyllysin chloromethyl ketone can also modulate inhibitory neurotransmission, contributing to a calming and anxiolytic effect on the nervous system. Together, these findings suggest that the polyherbal formulation has the potential to act as a nervine tonic through dual mechanisms: MAO-B inhibition to boost neurotransmitter levels, and GABA receptor modulation to promote relaxation and reduce anxiety. This dual action could make the formulation particularly effective in managing stress, anxiety, and nervous system-related disorders.

The nervines in the proposed novel polyherbal formulations have a long history of use in many different folk traditions for improving mental functioning, moods, and sleep. These herbs continue to be widely used in a similar fashion by most botanical practitioners and appear to be very safe and effective for addressing mild-to-moderate anxiety and its many symptoms. Hence, a detailed investigation of this formulation is mandatory before use.

ACKNOWLEDMENT: The authors are grateful to Central Sophisticated Instrumentation Facility, University of Calicut, Thenjipalam, for providing computational facilities for this work. We are thankful to Ayush Herbs Pvt Ltd, Himachal Pradesh, India. 176047 for sourcing the sample.

CONFLICT OF INTEREST: No Conflict of Interest.

REFERENCES:

1. Nervine Tonics_ Incredible Health Benefits, Uses and 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 eview 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. Free Radic Res 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 modi fi ed lignocellulosic fi bers 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 amlodipine besylate. 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 erlotinib 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
23. Jin Z, Mendu SK and Birnir B: GABA is an effective immunomodulatory molecule. Amino Acids 2013; 45(1): 87-94. doi:10.1007/s00726-011-1193-7
24. 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

How to cite this article:
Sherin S, Cleetus M, Dev J, Nsakkariya K and Hussan KPS: A unique polyherbal formulation for cognitive enhancement and mental health support. Int J Pharm Sci & Res 2025; 16(5): 1000-23. doi: 10.13040/IJPSR.0975-8232.16(5).1000-23.

Reviewer's recommendations:

1. Specify designation and current full address of corresponding author.
2. Check for spelling, grammar and punctuation error(s).