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Identification of different extracts and phytoconstituents of *Callistemon viminalis Cheel* for their anti-anxiety effects based on pharmacognostic, toxicological, and pharmacological strategies

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ABSTRACT

Background: Psychiatric disorders like depression and anxiety are global challenges, exacerbated by the limitations of synthetic medications, including addiction and toxic side effects.
Methods: This study meticulously investigated the pharmacognostic, phytochemical, toxicological, and pharmacological properties of Callistemon viminalis Cheel. Toxicological assessments, including hemocompatibility assays, LD_{50} studies, FOB analysis, biochemical parameters, and structural integrity of vital organs, were conducted on aqueous, methanolic, chloroform, and petroleum ether extracts of leaves and stems. Phytochemical profiling via qualitative tests and GC-MS screened extracts for molecular docking against key receptors. Categorically screened extracts were evaluated for therapeutic potential against LPS-induced anxiety in mice.
Results: Toxicological evaluations on experimental animals demonstrated the safety of various extracts, evidenced by no in vitro and in vivo toxicity. GC-MS identified numerous phytochemicals that passed "Lipinski's Rule of Five." These compounds were screened for molecular docking, revealing significant binding affinities with CB1, SERT, α2A-AR, and GABAβ2 receptors, suggesting potential therapeutic effects against anxiety. The phytoconstituents with the highest docking scores, particularly in aqueous and methanolic extracts, were further validated for their therapeutic efficacy. Preliminary analysis based on the EPM test and serum cortisol levels confirmed these extracts' superior therapeutic effectiveness.
Conclusion: In conclusion, aqueous and methanolic extracts of *Callistemon viminalis* Cheel's leaf and stem showed promising potential as therapeutic interventions for anxiety disorders.

1. Introduction

In this fast-paced world, a significant portion of the population struggles with psychiatric conditions (PC) such as depression, anxiety, and insomnia, posing substantial challenges globally. Major depressive disorders (MDD) are forecasted by the World Health Organization (WHO) to become the second most prevalent global health concern by 2020 [1]. Anxiety and depression prevalence surged by 25 % post-COVID-19, affecting 301 million people in 2019. The onset of anxiety often occurs during childhood or adolescence, emphasizing the importance of early intervention. These psychiatric disorders (PD) could sometimes provoke suicidal tendencies contribute to major health challenges and compromise the quality of life [2]. Anxiety is a prevalent mental health condition marked by excessive worry, fear, and nervousness, significantly impacting daily functioning. It encompasses disorders like generalized anxiety disorder (GAD), panic disorder, and social anxiety disorder. Despite genetic, environmental, and neurological factors contributing to its development, neurotransmitters such as gamma-aminobutyric acid (GABA), serotonin, and norepinephrine play pivotal roles in anxiety regulation [3]. GABA reduces neuronal excitability, while serotonin and norepinephrine regulate mood and stress responses. Dysregulation of serotonin transporters (SERT), alpha2A adrenergic receptors (α2A-AR), and cannabinoid receptors (CB1) are associated with increased anxiety [4,5]. The therapeutic strategy having significant competency in the regulation of these neurotransmitter-receptor interactions would be a promising approach

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for developing effective treatments.

The rising prevalence of mental health issues underscores the need for more effective treatments [6,7]. Complementary and alternative medicine (CAM), especially herbal remedies, has gained traction for PC [8,9]. Herbal medicine, rooted in tradition and natural sources, has historically addressed various ailments. Despite the reliance on non-Western practices by a substantial global population, many herbal resources remain untapped for their therapeutic potential [10,11]. One such resource is *Callistemon viminalis* cheel (CVC) or Weeping Bottlebrush, native to Australia's eastern coast, which holds cultural significance in Indigenous communities. While modern research explores its medicinal potential, including antibacterial, antifungal, and antioxidant properties, its full therapeutic capacity remains untouched [12,13]. In this study, we aim to provide a comprehensive investigation, including pharmacognostic, toxicological, and phytochemical analysis, which can significantly estimate the promising therapeutic effects of unexplored herbal extracts targeting specific receptor sites. The study includes the first-time reporting of diverse phytochemicals accessed by Gas chromatography-mass spectrometry (GC-MS), *in-vitro,* and *in-vivo* toxicological profiles of plant extracts. Furthermore, the therapeutic efficacy of bioactive phytoconstituents targeting CB1, SERT, α2A-AR, and gamma-aminobutyric acid receptor subunit beta-2 (GABAβ2) were examined by an *in-silico* and *in-vivo* approach. These findings shed light on its potential benefits and safety, thereby paving the way for prospective therapeutic utilization.

2. Materials and methods

2.1. Pharmacognostic analysis

2.1.1. Collection and identification of the plant

The leaves and stem of *CVC* were fetched from the Herbal Garden of Amity Institute of Pharmacy, Haryana, India, and authenticated by Dr. Sunita Garg Scientist, RHMA, CSIR-NIScPR, New Delhi, India on 12th May 2022.

2.1.2. Organoleptic and morphological analysis

Investigation of organoleptic and morphological properties of *CVC* leaf and stem were conducted, to gain insight into the sensory characteristics including appearance, odour, and flavour. Concurrently, a detailed examination of structural and physical features was conducted. The integration of organoleptic and morphological analyses aimed to provide a holistic view of disparate visual aspects and qualities of the plant parts [14]. This comprehensive approach yields valuable insights into the botanical specimen.

2.1.3. Physicochemical analysis

The physicochemical properties of *CVC* leaf and stem were evaluated by examining its ash content. The inorganic residue after the ignition process was conducted over the temperature range of 650–700 °C. This analysis estimates several ash parameters (Total ash percentages, acid-insoluble and water-soluble ash). Furthermore, moisture content was evaluated by calculating the weight loss during the ignition process [15]. Consecutively, water and alcohol solubility were determined with their extractive values. This thorough assessment provides the mineral content and overall composition of plant parts.

2.1.4. Preparation of leaf and stem extracts

The extraction of *CVC* (stems and leaves) was executed utilizing the hot percolation method (Soxhlet apparatus). The plant parts were washed, dried, and powdered carefully. The resulting powder was taken in beakers, each containing 500 ml of different solvents (petroleum ether, chloroform, distilled water, and methanol). Optimal extraction cycles were carried out at a moderate temperature range of 30–45 °C, retaining thermolabile compounds. Post extraction process, the extract solutions were filtered and concentrated at 50 °C using a rotary vacuum evaporator until a paste-like consistency was achieved. Subsequently, sample concentrations were adjusted to 100 μg/ml using their respective solvents.

2.2. Toxicological analysis

In vivo and in vitro studies were conducted to evaluate the adverse and toxic effects of the resulting extracts including aqueous, methanolic, chloroform, and petroleum ether from the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*. This approach conceded a systematic examination of possible harmful effects on living organisms.

2.2.1. In vitro study

2.2.1.1. Assessment of heavy metals. One gram of powdered samples of *CVC* (leaf and stem) was placed in separate beakers, containing 15 ml of nitric acid (HNO_3) and 5 ml of perchloric acid ($HClO_4$). These mixtures were then heated incrementally from 90°C to 170°C. At 170°C, hydrogen peroxide (H_2O_2) was added until white fumes appeared, indicating the reaction's completion. After cooling, the solutions were diluted up to 50 ml with deionized water and filtered through the Whatman No. 42 filter paper. The resulting solutions were analyzed for heavy metals using coupled plasma-atomic emission spectrometry (ICP-OES) [16].

2.2.1.2. Hemocompatibility assay. The Hemolytic assay was carried out by taking (100 μL) fresh blood samples collected from mice and incubated with normal saline, Triton X (standard reagent), and various extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* for 1 hour under controlled conditions (5 % CO_2, 37°C, 95 % humidity). Absorbance was measured at 450 nm to calculate the hemolytic rate [17].

$$\text{Hemolytic rate}(\%) = \frac{\text{Sample absorbance} - \text{Negative control absorbance}}{\text{Positive control absorbance} - \text{Negative control absorbance}} \times 100$$

Further RBC aggregation assay was conducted which involved centrifuging fresh blood at 2000 g for 10 minutes, resuspending the blood pellets in normal saline, and incubating them with plant extracts [18]. Smears from these mixtures were microscopically examined.

2.2.2. In vivo study

2.2.2.1. Lethal dose (LD_{50}). The LD_{50} of various extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* were studied to assess the dose-dependent lethal impact of extracts. This experiment involved a total of 8 groups (for each extract) which carried 18 animals (albino mice (20–25 g) of either sex) in each group. Mice of each group were randomly divided into another six groups that received different doses of 10, 100, 1000 1600, 2900, and 5000 mg/kg of different extracts of leaf (AqCL, MeCL,

ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*. The LD_{50} was calculated as the dose of any individual extract that can cause 50 % mortality of mice in specific groups, within a specified period of 21 days to report any delayed effect [19].

2.2.2.2. Assessment of toxicity based on behavioral analysis, oxidative stress level, and histology. Further, toxicology analysis of different extracts of the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* was performed on experimental albino mice of either sex, approved by the "Institutional Animal Ethics Committee" following CPCSEA's guidelines (Approval No. CPCSEA/IAEC/07/202/65). A total of 54 mice (20–25 g) were acclimatized and divided into 9 groups (n=6) including normal control and eight test groups (receiving normal saline and 180 mg/kg of various extracts daily for two weeks respectively). This selection of dose was based on a widely recognized protocol of the OECD (Organisation for Economic Co-operation and Development) guidelines =423 for the testing of chemicals for sub-chronic toxicity studies [20].

Behavioural changes were monitored using the functional observational battery (FOB) to ascertain immediate adverse effects post-oral administration of extracts from *CVC* leaves (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) [21]. Treated animals were compared to a control group to detect any immediate reactions. Long-term adverse effects based on oxidative stress were assessed through biochemical measurements including reduced glutathione (GSH) levels, superoxide dismutase (SOD) activity, catalase (CAT) activity, and thiobarbituric acid reactive substances (TBARS) assay as described previously [22]. Moreover, any structural alteration in the vital organs (liver, kidneys) induced by this oral administration of different extracts of leaf and stem was accessed by tissue histology [15,23]. In-brief, vital organs were isolated from each group, preserved in 10 % formalin, encased in paraffin wax, sectioned, and stained with hematoxylin and eosin for histological analysis under a light microscope. These comprehensive assessments provided insights into the safety, toxicity, and biochemical impacts of *CVC* extracts.

2.3. Assessment of phytoconstituents

2.3.1. Primary and secondary phytochemical metabolites

Each extract of *CVC* was analyzed for the presence of various compounds, including alkaloids, polyphenols, glycosides, anthraquinone glycosides, phytosterols, amino acids, and proteins, saponins, flavonoids, tannins, steroids, carbohydrates, and gums and mucilages, using specific reagents. In brief, the phytoconstituents in *CVC* were detected using various methods including Molisch's test for carbohydrates, Ferric chloride assay for tannins, Folin-Ciocalteu assay for phenolic compounds, Shinoda test for flavonoids, Salkowski test for plant sterols, and Dragendorff's reagent for alkaloids [14].

2.3.2. Identification of bioactive phytoconstituents using GC-MS analysis

A qualitative analysis of various extracts from *CVC* of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) was performed to identify phytochemicals using GC-MS at Jawaharlal Nehru University Delhi, India. Extracts were dissolved in petroleum ether, chloroform, and methanol, while the aqueous extract was dissolved in methanol with a pinch of anhydrous sodium sulphate to ensure a particulate-free solution. This solution was stirred vigorously for 10 seconds using a vortex stirrer and filtered through a 0.2-micron membrane filter for GC-MS analysis.

The GC-MS analysis was conducted using a GC-MS-QP-2010 Plus instrument with electron ionization at 70 eV. Helium gas (99.99 %) served as the carrier at a 1.21 ml/min flow rate. A 1 μL extract sample was injected with a split ratio of 10:1, at an injector temperature of 260℃ and an ion source temperature of 220℃. Naphthalene was used as the standard for the analysis. Each sample was analyzed for approximately 66.48 minutes. The resulting spectra were compared with the NIST 17 library, which includes over 6200 patterns. The retention time, molecular formula, and molecular weight of the compounds were recorded [24].

2.4. Molecular docking (MD) analysis

2.4.1. Selection of bioactive compounds (based on "Lipinski rule")

By applying Lipinski's rule of five on the list of phytochemical compounds derived from the GC-MS analysis of the extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*. It identified the bioactive compounds based on their molecular weight, log P value, and the number of hydrogen bond donors and acceptors. This rule aids in scrutinizing and filtering the comprehensive list of phytochemical compounds [14].

2.4.2. Molecular interaction of the selected bioactive compound with CB1

MD was utilized to determine the finest alignment of ligands and proteins. Selected bioactive compounds of the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* were further analyzed by MD to detect interaction scores with the targeted protein (CB1). The glide module of Schrodinger's Maestro molecular modeling suite release 2023–24 was used for MD interaction between ligand (Selected bioactive phytoconstituents of leaf extracts (AqCL, MeCL, ChCL, and PtCL, respectively) and stem extracts (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*) and protein (CB1). The ligand structures were retrieved from the PubChem compound database at NCBI (http://pubchem.ncbi.nlm.nih.gov/) and underwent preparation via the LigPrep module to generate a 3D structure and minimize the confirmational energies.

In this study, the CB1 protein (PDB ID: 5TGZ) was chosen initially due to its significant role in anxiety. CB1 is a protein encoded by the CNR1 gene in humans. It is an integral component of the endocannabinoid system, responsible for modulating neurotransmitters such as serotonin and GABA, thus exerting influence over emotional processing [25]. The interactions between protein and bioactive compounds extracted from the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* were examined individually. These proteins' 3D X-ray crystal structures were obtained from the Protein Data Bank (PDB). These proteins are believed to play a pivotal role in the development and progression of neurodegenerative disorders.

2.4.3. Preparation of target protein and docking

The Schrodinger's Maestro molecular modeling suite was employed to process and enhance the protein structures, refining the initial raw PDB structures into more accurate protein models through the protein preparation wizard module. This refinement process also included the elimination of any water molecules originally present in the structures. Subsequently, Schrodinger's Maestro software facilitated a comprehensive analysis of the protein structures, examining structural elements, hydrogen bond interactions, and non-bond interactions between ligands and active site residues, and generating high-quality visual representations of these findings.

For the docking process, both the prepared ligands and target proteins underwent analysis using the protein preparation wizard module. During this process, multiple conformations for the ligands were generated, and final energy refinement was performed to ascertain the optimal ligand pose. Following this, the docking scores for the best ligand poses with their respective target proteins were calculated for all tested bioactive compounds.

2.4.4. Molecular interaction with SERT, α2A-AR, and GABAβ2

In addition to the CB1, SERT, α2A-AR, and GABAβ2 play another prominent role in anxiety. The dysregulation of GABAβ2 receptors, alterations in SERT function, and the modulation of norepinephrine

Table: 1
Organoleptic, morphological property, physiochemical parameters, and yield percentage of different extracts of *Callistemon viminalis* leaves and stem.

Organoleptic properties			
S.N.	**Parameter**	**Leaf**	**Stem**
		Inference	**Inference**
1.	Color	Green	Greenish grey
2.	Odors	pleasant	pleasant
3.	Taste	Bitter	Bitter
4.	Shape	Elliptical	Cylindrical
5.	Texture	Smooth and shiny	Rough to touch
Morphological properties of leaf			
1.	Length	7.1-7.3 cm	
2.	Breath	0.4-0.6 cm	
3.	Base	Rounded	
4.	Veins	Reticulate	
5.	Apex	sharp	
6.	Margin	Entire	
7.	Lamina	Dark green	
8.	Venation	Pinnate	
Physiochemical parameters of *Callistemon Viminalis* leaves and stem			
S.N.	**Parameter**	***Callistemon Viminalis* Leaves (%w/w)**	**Callistemon *Viminalis* Stem (%w/w)**
1.	Loss on drying	3.2±0.26% w/w	3.5±0.23% w/w
2.	Total Ash Content	4.74±0.27% w/w	7.3±0.5% w/w
3.	Acid-insoluble ash value	2.4±0.06% w/w	3.4±0.6% w/w
4.	Water soluble ash value	2.32±0.17% w/w	2.5±0.13% w/w
5.	Forming Index	Less than 100	Less than 100
6.	Swelling Index	NIL	NIL
7.	Crude fiber content	13.3±0.1	15.3±0.5
8.	Volatile oil content	17.6±0.4	11.5±0.5
9.	Alcohol soluble extractive value	14.3±0.5% w/w	12.4±0.6% w/w
10.	Water soluble extractive value	11.5±0.6% w/w	12.2±0.7% w/w
The Yield percentage of different extracts of leaf and stem of Callistemon viminalis.			
S.N.	**Extract**	**Callistemon *viminalis* Leaves Extractive Value**	**Callistemon *viminalis* Stem Extractive Value**
1.	**Pet. Ether**	2.4%	2.2%
2.	**Chloroform**	3.4%	3.4%
3.	**Methanol**	8.56%	7.59%
4.	**Aqueous**	6.07%	5.58%

release via the α2A-AR collectively highlight the potential contribution to the range of neurological and PC including anxiety. Thus, the selected bioactive phytoconstituents of leaf extracts (AqCL, MeCL, ChCL, and PtCL, respectively) and stem extracts (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* (top 10 compounds from each sample having the highest docking score with CB1) were further studied for molecular interaction with these proteins. The PDB ID of these proteins includes SERT (PDB ID: 6AWO), α2A adrenergic receptor (PDB ID: 6KUY), and GABAβ2 (PDB ID:6X3X). Following the analysis of the interaction between the bioactive compounds (top 10 molecules from each extract having the highest docking score with CB1) were further employed for subsequent docking experiments with two distinct proteins, namely SERT, α2A-AR and GABAβ2 by following the same process as mentioned above for the protein CB1.

2.5. Preliminary validation of extracts for therapeutic effect

For evaluating and validating the predictable efficacy of various extracts through the in-silico MD method, preliminary investigations were conducted using lipopolysaccharide (LPS)-induced anxiety model in mice. A total of 66 mice (20–25 g) were randomly divided into 11 groups (n=6 each). These groups included a normal control group that received normal Saline orally, a disease control group that received 0.5 mg/kg/day intraperitoneal LPS for 7 days before the experiment to induce anxiety, and a standard control group treated with 0.75 mg/kg diazepam intraperitoneally 30 minutes before the experiment. The remaining eight test groups received various plant part extracts at a dosage of 180 mg/kg.

Behavioral changes were assessed using the elevated plus maze

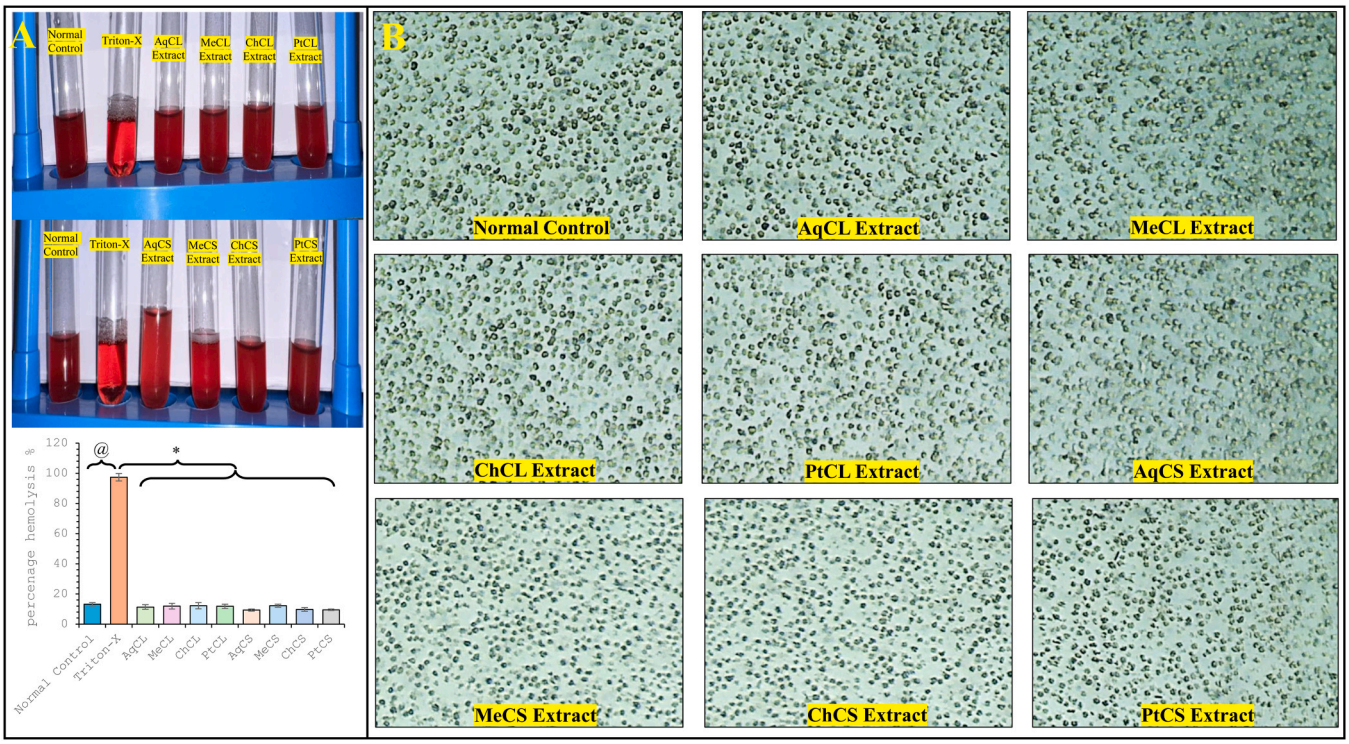

Fig. 1. : The figure represents the effect of *Callistemon viminalis cheel* extracts (leaf and stem) on the hemolytic rate **(A)**, on the RBC agglutination rate **(B)**. Aqueous extract, methanolic extract, chloroform extract and petroleum ether extract of leaf and stem has been represented as AqCL, MeCL, ChCL, PtCL for leaf and AqCS, MeCS, ChCS, and PtCS for stem, respectively. All values were expressed as mean ± SD whereas, '@' represents $p < 0.05$ vs Normal Control; '*' represents $p < 0.05$ vs Triton X treated group.

(EPM), which consists of two open arms (50 × 10 cm) and two closed arms (50 × 40 × 10 cm), elevated 50 cm above the floor. It features an open ceiling at 50 cm of height above the floor helps in assessing anxiety-like behavior in mice. The mice underwent EPM testing 60 minutes after the administration of the various doses. Each mouse was gently placed in the center of the EPM with its head oriented toward an open arm. The behavioral activities of mice were assessed based on two criteria during the 5-minute test period. (a) the frequency of entries into the open arms and (b) the duration of time spent in these arms [24, 26,27].

Upon completion of the behavioral experiment, blood samples were collected via retro-orbital puncture, followed by the separation of serum samples through centrifugation (6000 rpm, 20 minutes). These samples were then stored at −80°C for subsequent analysis. Serum cortisol levels were evaluated using commercially available enzyme-linked immunosorbent assay (ELISA) kits, by established protocols [28].

2.6. Statistical analysis

The data obtained are expressed in the format of average ± SD and analysed in version 4.00 of the Graphic Pad Prism software. Data statistical analysis is carried out in the significance of difference was determined by one-way ANOVA. p-values less than 0.05 ($p < 0.05$) were considered statistically significant.

3. Results

3.1. Pharmacognostic evaluation

3.1.1. Authentication and evaluation of organoleptic property

CVC leaf and stem were collected and authenticated by Chief Scientist Dr. Sunita Garg (RHMD, CSIR-NISCAIR) under the authentication numbers *NIScPR/RHMD/Consult/2022/4106–07* and *NIScPR/RHMD/Consult/2022/4084–85* for leaf and stem, respectively. According to the organoleptic and morphological characteristics, the stem and leaf of *CVC* exhibit distinct features. The leaves of *CVC* were green, and elliptical, with a smooth and shiny texture. They measure 0.4–0.6 cm in width and 7.1–7.3 cm in length, have a rounded base, a sharp apex, entire margins, a dark green lamina, and pinnate venation. The stem was cylindrical, and rough, and presented a greenish-grey coloration. Both the stem and leaves share a pleasant aroma but possess a bitter taste as shown in Table 1.

3.1.2. Evaluation of physicochemical parameters (determination of ash value)

The moisture content in the leaf and stem powder of *CVC* was recorded as 3.2±0.26 % w/w and 3.5±0.23 % w/w, respectively (within the acceptable range of 5–8 %), thus this form could be favorable for storage for longer duration. The total ash values were 4.74 ±0.27 % w/w for the leaf and 7.3±0.5 % w/w for the stem, which were within permissible limits, suggesting an absence of impurities. The low acid-insoluble ash values and water-soluble ash values including 2.4 ±0.06 % w/w for the leaf, 3.4±0.6 % w/w for the stem, and 2.32 ±0.17 % w/w for the leaf, 2.5±0.13 % w/w for the stem, respectively indicated a lack of adulteration and suggested minimal interference with gastrointestinal absorption [29]. Physical properties, including the foaming index, swelling index, and crude fiber content, were evaluated. The foaming index showed no significant difference between the stem and leaf, suggesting the presence of saponins [29]. A low swelling index indicated minimal amounts of mucilage, pectin, and hemicellulose. The crude fiber content was higher in the stem (15.3±0.5) than in the leaf (13.3±0.1), indicating greater amounts of cellulose, hemicellulose, and lignin (Table 1). The total volatile oil content, an important quality indicator, was found to be 17.6±0.4 in the leaf and 11.5±0.5 in the stem, demonstrating a higher concentration in the leaf. The alcohol-soluble extractive values for the leaves and stems were observed as 14.3

Table: 2
Assessment of Acute toxicity test of petroleum ether, chloroform, methanolic, and aqueous extracts of leaf and stem of Callistemon viminalis cheel including mortality (%) and LD_{50}.

S. N.	Does (mg/kg)	10 mg		100 mg		1000 mg		1600 mg		2900 mg		5000 mg		LD_{50} Value (mg/kg)
	Plant extract	Log10 Dose	% Mortality	Log10 Dose	% Mortality	Log10 Dose	% Mortality	Log10 Dose	% Mortality	Log10 Dose	% Mortality	Log10 Dose	% Mortality	
Leaf extracts														
1	Petroleum ether extracts	1	0	2	0	3	0	3.2	33.3	3.5	66.7	3.7	100	1862
2	Chloroform extracts	1	0	2	0	3	0	3.2	33.3	3.5	66.7	3.7	100	1862
3	Methanolic extracts	1	0	2	0	3	0	3.2	33.3	3.5	66.7	3.7	100	1862
4	Aqueous extracts	1	0	2	0	3	0	3.2	66.7	3.5	66.7	3.7	100	2290
Stem extracts														
5	Petroleum ether extracts	1	0	2	0	3	0	3.2	33.3	3.5	66.7	3.7	100	1862
6	Chloroform extracts	1	0	2	0	3	0	3.2	33.3	3.5	33.3	3.7	100	3890
7	Methanolic extracts	1	0	2	0	3	0	3.2	33.3	3.5	66.7	3.7	100	1862
8	Aqueous extracts	1	0	2	0	3	0	3.2	33.3	3.5	66.7	3.7	100	1862

±0.5 % w/w and 12.4±0.6 % w/w, respectively, while the water-soluble extractive values were measured as 11.5±0.6 % w/w for the leaf and 12.2±0.7 % w/w for the stem. The higher alcohol-soluble extractive value suggests that alcohol is a more effective solvent for extraction as shown in Table 1.

3.1.3. Evaluation of leaf and stem extracts yields

The extraction utilized the hot percolation method (Soxhlet apparatus), maintaining a controlled temperature range of 30–45 °C to prevent the degradation of thermosensitive compounds in the leaf and stem. Dried leaf and stem samples (50 g each) were subjected to extraction using various solvents: petroleum ether, chloroform, distilled water, and methanol. The resulting percentage yields for the leaf were 2.1 %, 3.4 %, 6.07 %, and 8.56 %, respectively, while the yields for the stem were 2.2 %, 3.4 %, 5.58 %, and 7.59 %, respectively (Table 1).

3.2. In-vitro assessment of the safety and toxicological profile

3.2.1. Evaluation of heavy metals toxicity

The presence of heavy metals such as lead, cadmium, arsenic, and mercury in plants poses significant health risks upon consumption, as these toxic metals can accumulate in edible plant parts and cause various health issues. Lead can impact the nervous system, cadmium is a known carcinogen causing kidney and respiratory problems, arsenic contributes to skin lesions and cardiovascular diseases, and mercury affects the nervous system [30]. Our analysis indicates that the plant does not contain any hazardous metals, underscoring it as a safer option. Adhering to safety standards, it is crucial to prevent the accumulation of unsafe metal levels in the human body, thereby ensuring overall health protection and endorsing the safety of different extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* for consumption.

3.2.2. Evaluation of hemolytic rate and agglutination

The study evaluated the impact of foreign materials on the circulatory system by assessing hemolysis rates after 1-hour incubation with Triton X and various extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*, in comparison to normal saline. The findings revealed a significant increase in hemolysis with Triton X compared to normal saline. However, exposure to different *CVC* extracts did not lead to a notable rise in hemolysis, remaining below the permissible limit of 5 % [31,32]. Moreover, no disturbances in RBC morphology or agglutination were observed, further affirming the blood compatibility of *CVC* extracts for potential biological applications without adverse effects (Fig. 1). These consequences suggest that *CVC* extracts of leaf and stem are non-toxic, hemocompatible, and offer protection against hemolysis.

3.2.3. In-vivo assessment of the safety and toxicological profile

3.2.3.1. Evaluation of LD_{50}. The toxicological screening of *CVC* was essential to ensure the safety and efficacy of extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively). During the assessment, signs of toxicity were observed in the test groups based on their LD_{50} value. LD_{50} quantifies a substance's acute toxicity by determining the lethal dose for 50 % of a test population, aiding comparative toxicity and indicating relative hazard levels. The different extracts of the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) of *CVC* exhibited acute toxicity with LD_{50} values of 2290, 1862, 1862, and 1862 mg/kg, respectively. Moreover, the stem extracts (AqCS, MeCS, ChCS, and PtCS, respectively), showed the LD_{50} values of 1862, 1862, 3890, and 1862 mg/kg, respectively (Table 2). These LD_{50} values were calculated based on the coefficient of determination or the proportion of the variance in the dependent variable (dose). These findings suggest that the extracts have the potential for

Table: 3
Toxicological evaluations of the various extracts of leaf and stem of Callistemon viminalis cheel based on the estimation of functional observational battery (FOB).

Sr. No	Parameter	Normal control	AqCL	MeCL	ChCL	PtCL	AqCS	MeCS	ChCS	PtCS
Home Cage										
1	Spontaneous activity level	3	3	3	3	3	3	3	3	3
2	Respiration	3	3	3	3	3	3	3	3	3
3	Convulsions	A	A	A	A	A	A	A	A	A
4	Tremors	A	A	A	A	A	A	A	A	A
5	Fasiculations	A	A	A	A	A	A	A	A	A
6	Tonus	A	A	A	A	A	A	A	A	A
7	Clonus	A	A	A	A	A	A	A	A	A
8	Vocalization	A	A	A	A	A	A	A	A	A
9	Straubs tail	A	A	A	A	A	A	A	A	A
10	Writhing	A	A	A	A	A	A	A	A	A
11	retropulsion	A	A	A	A	A	A	A	A	A
12	Diarrhoea	A	A	A	A	A	A	A	A	A
Handheld										
13	Excitation	2	1	2	3	3	2	2	2	3
14	Salivation	0	0	0	0	0	0	0	0	0
15	Lacrimation	0	0	0	0	0	0	0	0	0
16	Piloerection	A	A	A	A	A	A	A	A	A
17	fur appearance	A	A	A	A	A	A	A	A	A
18	Ptosis	A	A	A	A	A	A	A	A	A
19	Exophthalmia	A	A	A	A	A	A	A	A	A
Open Cage										
20	Supported rears	5	5	4	5	5	4	4	4	5
21	Unsupported rears	0	0	0	0	0	0	0	0	0
22	Spontaneous activity level	3	2	3	3	4	4	3	4	4
23	Gait	1	1	1	1	1	1	1	1	1
24	Arousal	4	3	4	4	4	4	4	4	4
25	Convulsions	A	A	A	A	A	A	A	A	A
26	Straubs tail	A	A	A	A	A	A	A	A	A
27	Writhing	A	A	A	A	A	A	A	A	A
28	Retropulsion	A	A	A	A	A	A	A	A	A
29	Stereotypy	A	A	A	A	A	A	A	A	A
30	Diarrhoea	A	A	A	A	A	A	A	A	A
31	Auditory response	3	3	3	3	3	3	3	3	3
32	Visual approach	P	P	P	P	P	P	P	P	P
33	Olfactory response	P	P	P	P	P	P	P	P	P
34	Pinna reflex	P	P	P	P	P	P	P	P	P
35	Extensor reflex	P	P	P	P	P	P	P	P	P
36	Palpebral reflex	P	P	P	P	P	P	P	P	P
37	Visual placing	P	P	P	P	P	P	P	P	P
38	Surface righting	P	P	P	P	P	P	P	P	P
39	Aerial righting	P	P	P	P	P	P	P	P	P
40	Tail pinch response	P	P	P	P	P	P	P	P	P

development into pharmacological agents to treat various ailments.

3.2.4. *Evaluation of functional observational battery (FOB)*

The initial assessment of the central nervous system (CNS) was based on the analysis of FOB to detect any sudden behavioral changes suggestive of potential substance toxicity. Various behavioral tests were conducted on animals exposed to distinct extracts of the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*. Post-oral administration of these extracts (180 mg/kg, single dose) showed no abnormal behaviors such as convulsions, tremors, vocalizations, or postural alterations noted during home cage observation. Additionally, external observations and handling of the animals revealed no significant behavioral deviations. Similarly, the open-field activities were further assessed to validate the absence of behavioral alterations, confirming the overall safety profile of *CVC* extracts (Table 3).

3.2.5. *Evaluation of oxidative marker*

The human body's defense against oxidative stress includes GSH, a potent antioxidant comprising cysteine, glycine, and glutamate. GSH mitigates oxidative stress, preventing tissue damage. TBARS assay assesses lipid peroxidation, while enzymes like CAT and SOD neutralize reactive oxygen species, crucial for understanding the oxidative defense and extract effects. Evaluation of these parameters in brain tissue showed no significant alterations, reinforcing that the tested that the extracts of leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* do not detrimentally affect the body's intrinsic antioxidant defense mechanisms (Fig. 2).

3.2.6. *Evaluation of histological analysis*

The microscopic examination of histological sections from vital organs enables a detailed assessment of any abnormalities or adverse effects induced by a test drug. Vital organs from all mice groups treated with leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC*, were analyzed based on histology of H and E staining and observed at 40× magnification to detect potential damage or injury. The histological analysis indicated that the structural arrangements of organs in the pre-treated groups (administered of various extracts (180 mg/kg/day, *p.o*) for 14 days) exhibited insignificant alterations compared to the normal control group. Photomicrographs revealed negligible disruptions in the central vein and hepatocytes of the liver, and no discernible changes in the parenchyma, tubules, and glomeruli of the kidney, compared to the histological sections of normal control mice. These findings unequivocally demonstrate the non-toxic nature of all *CVC* extracts of leaf and stem across vital organs (Fig. 3).

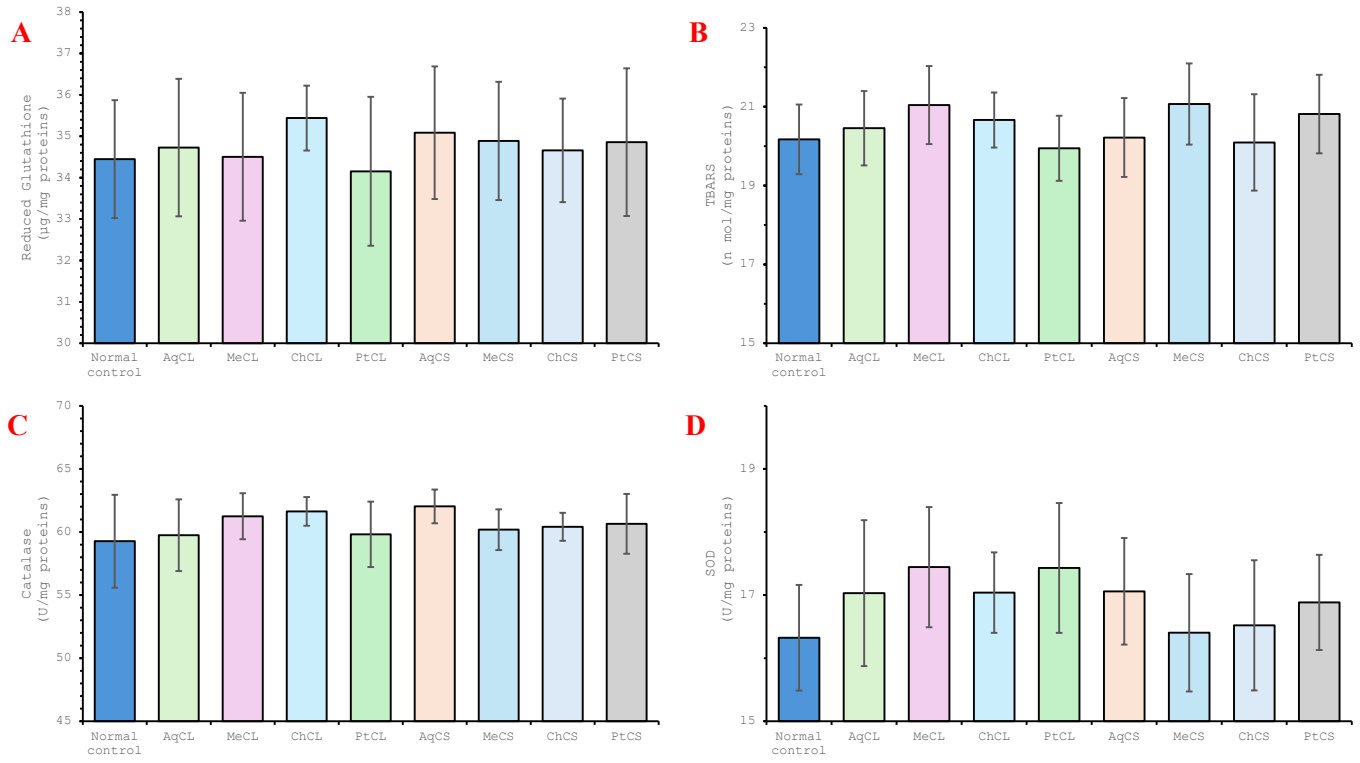

Fig. 2. : The figure describes the effect of *Callistemon viminalis* cheel extracts (leaf and stem) on the biochemical oxidative markers including reduced (A) GSH level (µg/mg protein), **(B)** TBARS (nmol/mg protein), **(C)** CAT (U/mg protein), and **(D)** SOD (U/mg protein). All values were expressed as mean ± SD whereas, '@' represents $p < 0.05$ vs Normal Control; '*' represents $p < 0.05$ vs disease control group.

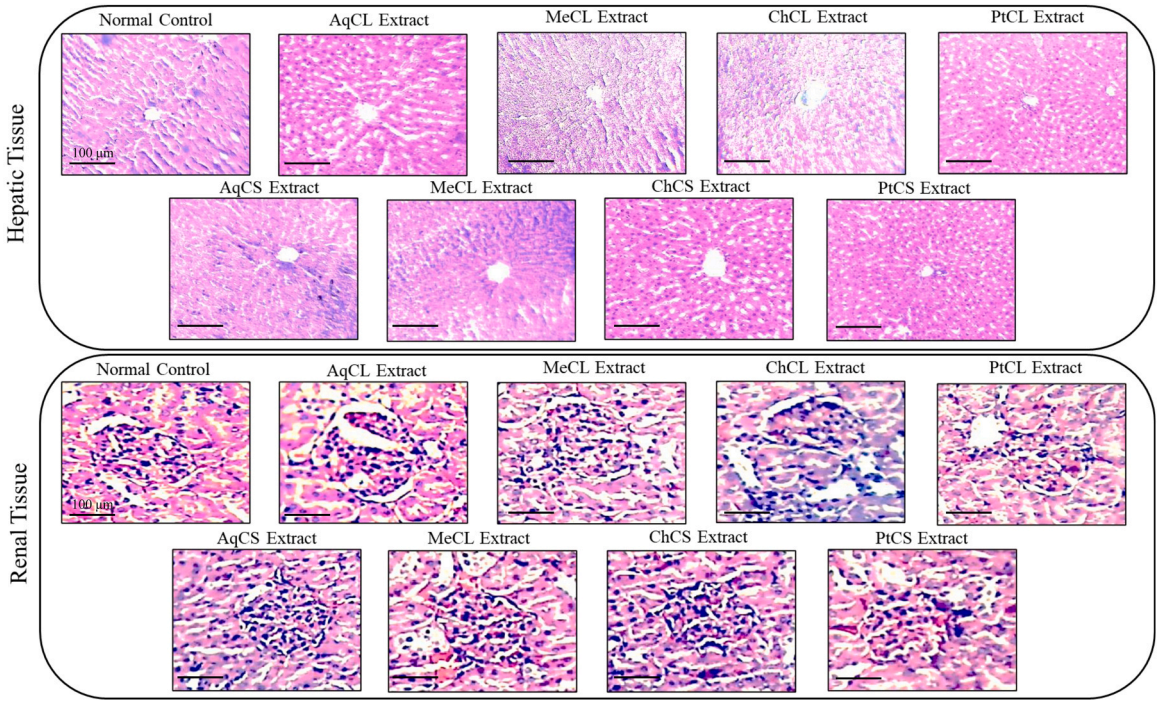

Fig. 3. : Structural integrity and cells/tissues arrangements were shown by these histological photomicrographs of vital organs including hepatic and renal tissue against treatment of the *Callistemon viminalis cheel*. The histological examination was performed using Motic Microscope BA310 (Motic, USA) at 40× (scale bar=100 µm).

Table: 4
Phytochemical screening of different extracts of *Callistemon viminalis cheel* leaves and stem.

Sr. No.	Phytoconstituents	Extracts							
		Callistemon *viminalis* leaves				Callistemon *viminalis* stem			
		PeCL	ChCL	MeCL	AqCL	PeCS	ChCS	MeCS	AqCS
1.	Carbohydrates	–	–	++	+	–	–	++	+
2.	Tannins and Phenols	–	–	++	++	–	–	++	++
3.	Saponins	–	–	++	++	–	–	++	++
4.	Glycosides	–	–	++	++	–	–	++	++
5.	Anthraquinone glycoside	–	–	++	++	–	—	++	++
6.	Flavanoids	++	++	++	++	++	++	++	++
7.	Sterols	++	–	++	++	++	–	++	+
8.	Phytosterol	++	–	++	+	++	–	++	+
9.	Alkaloids	–	–	+	+	–	–	+	–
10.	Amino acids and Proteins	–	–	–	–	–	–	–	–
11.	Gum and Mucilage	–	–	–	–	–	–	–	–

3.3. Phytochemical analysis

3.3.1. Evaluation of primary and secondary phytochemical metabolites

The therapeutic efficacy of medicinal plants depends heavily on secondary metabolites, including flavonoids, saponins, phytosterols, alkaloids, glycosides, tannins, phenols, and sterols. Analysis of *CVC* extracts consistently reveals various compounds. The analysis of 1° and 2° phytochemical metabolite reveals a rich presence of flavonoids across all extracts, with significant amounts of tannins, phenols, saponins, glycosides, and anthraquinone glycosides in methanol and aqueous extracts. Sterols and phytosterols are also prevalent in petroleum ether and methanol extracts (Table 4). Alkaloids are less common, appearing only in methanol and aqueous extracts of leaves and methanol extracts of stems. Amino acids, proteins, gums, and mucilage are notably absent in all tested extracts. This phytochemical profile suggests that *CVC* leaves and stems have potential medicinal properties attributed to the identified constituents.

The bioactive compounds in *CVC* highlight its potential for addressing health conditions and diverse therapeutic applications. Carbohydrates serve as essential energy sources. Tannins and phenolic compounds offer therapeutic potential for skin disorders, wounds, and inflammatory conditions, and their antioxidant properties benefit vascular and cardiac health [33]. Saponins, glycosides, and anthraquinone glycosides contribute to specific health benefits. Flavonoids exhibit anti-inflammatory and anti-hyperglycemic properties [34]. Plant sterols lower LDL cholesterol, reduce cardiovascular risk, and modulate insulin sensitivity [8]. Alkaloids provide analgesic, anti-inflammatory, antimicrobial, and antioxidant effects, with potential applications in cancer treatment and respiratory support [35].

3.3.2. Evaluation of GC-MS analysis for phytochemical assessment

The evaluation of the chemical structure and composition of the extract unveils its biological potential. Remarkably, prior studies have not reported the characterization of phytochemicals in the various extracts of the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* using GC-MS equipment. Our study addresses this gap, presenting results from GC-MS analysis revealing 38, 60, 42, and 71 phytoconstituents in the different extracts (AqCL, MeCL, ChCL, and PtCL, respectively) of leaf, and 44, 65, 23, and 81 phytoconstituents in the different extracts (AqCS, MeCS, ChCS, and PtCS, respectively) of stem (Supplementary Tables 1 and 2).

These analyses furnish detailed information on the active principles, including their molecular formula, molecular weight, and retention index of phytoconstituents in the Stem and leaf extracts. Additionally, the study of extractive value provides valuable insights and can establish suitable standards for determining the quality of plant material in future investigations. Proximate analysis of the data reveals the composition of

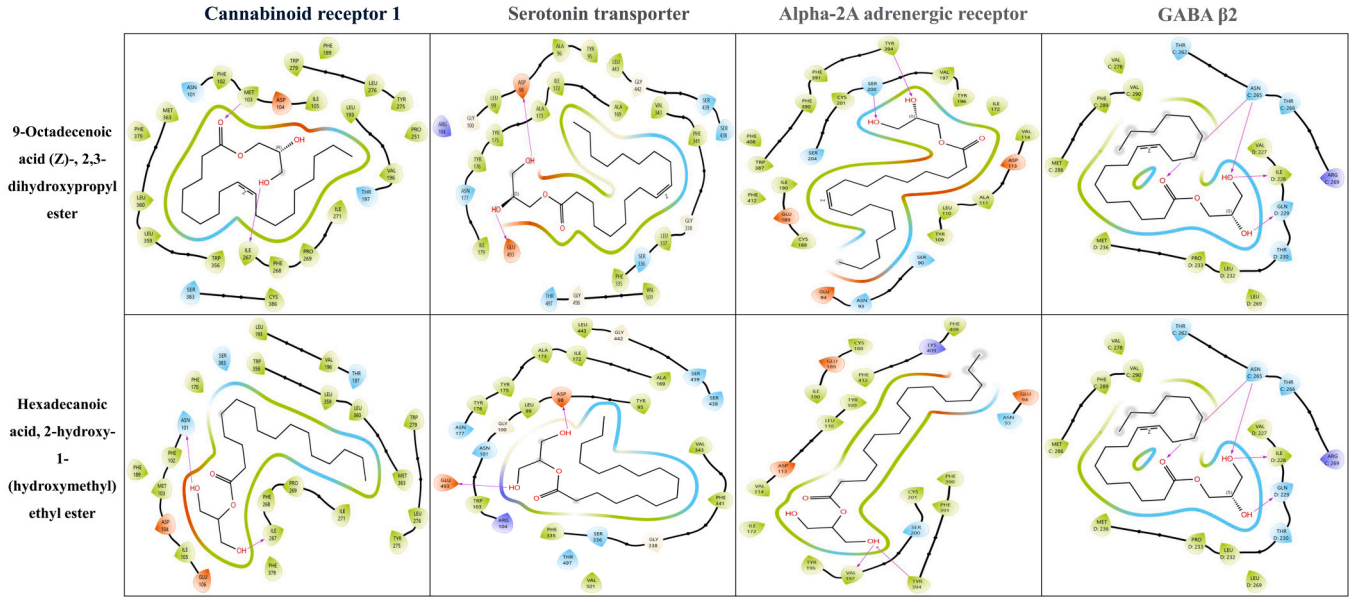

Fig. 4. : The figure portrays the molecular interactions between the selected phytoconstituents (9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester) of AqCL extract of leaf of *Callistemon viminalis cheel* with CB1, SERT, α2A-AR, and GABAβ2.

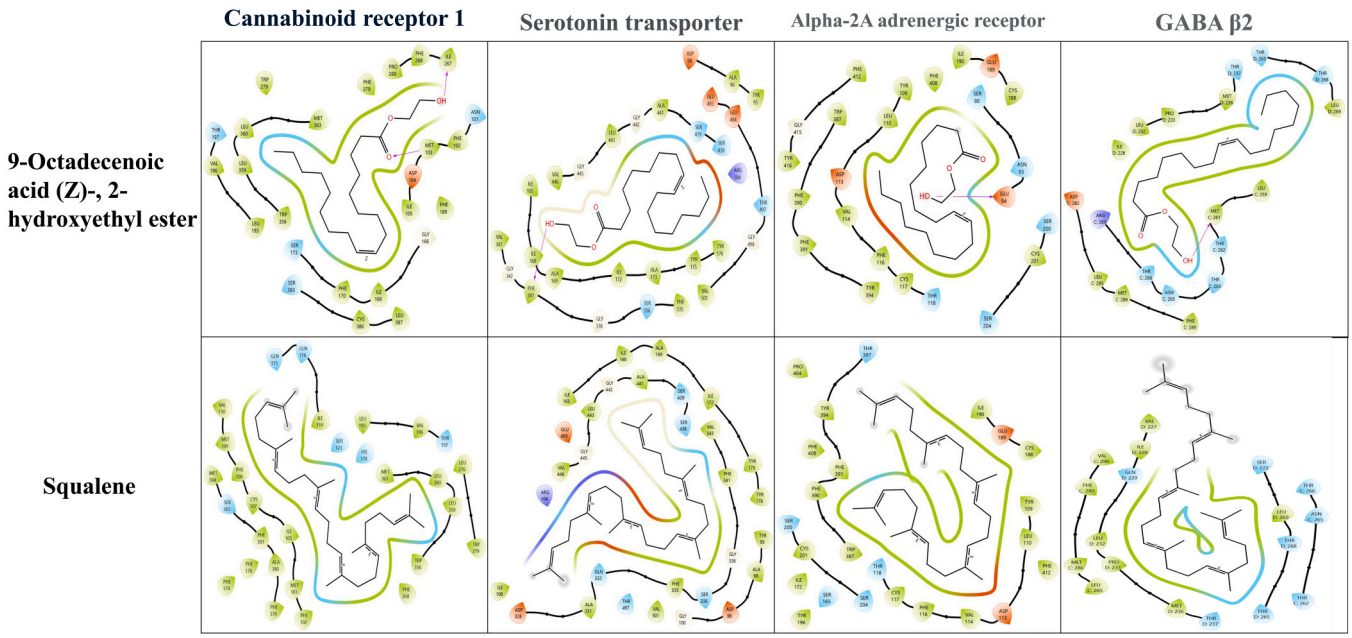

Fig. 5. : The figure shows the molecular interactions between the selected phytoconstituents (9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester, and Squalene) of MeCL extract of leaf of *Callistemon viminalis cheel* with CB1, SERT, α2A-AR, and GABAβ2.

the different extracts from the stem and leaf of *CVC*, and the detail of phytoconstituents has been introduced.

3.3.3. Screening of bioactive compounds based on 'Lipinski's Rule of Five'

The screening of bioactive phytoconstituents was performed using Schrödinger's maestro software suite. From the plethora of phytoconstituents of the leaf (AqCL, MeCL, ChCL, and PtCL, respectively) and stem (AqCS, MeCS, ChCS, and PtCS, respectively) of *CVC* were identified via GC-MS results and a subset of bioactive compounds was selected based on adherence to Lipinski's Rule of Five. This well-established rule in drug discovery assists in assessing the drug-likeness of chemical compounds, predicting their potential as drug candidates, and evaluating oral bioavailability. Lipinski's Rule of Five criteria included molecular weight, lipophilicity (LogP), hydrogen bond donors, and acceptors.

According to these criteria, we selectively identified 22, 23, 19, and 20 bioactive phytoconstituents (out of a total of 38, 60, 42, and 71) in the AqCL, MeCL, ChCL, and PtCL extracts of the leaf, respectively. Similarly, 24, 29, 11, and 29 phytoconstituents (out of a total of 44, 65, 23, and 81) were selected from the AqCS, MeCS, ChCS, and PtCS extracts of the stem, respectively. Detailed information regarding the selected bioactive phytoconstituents from both the leaf and stem, including

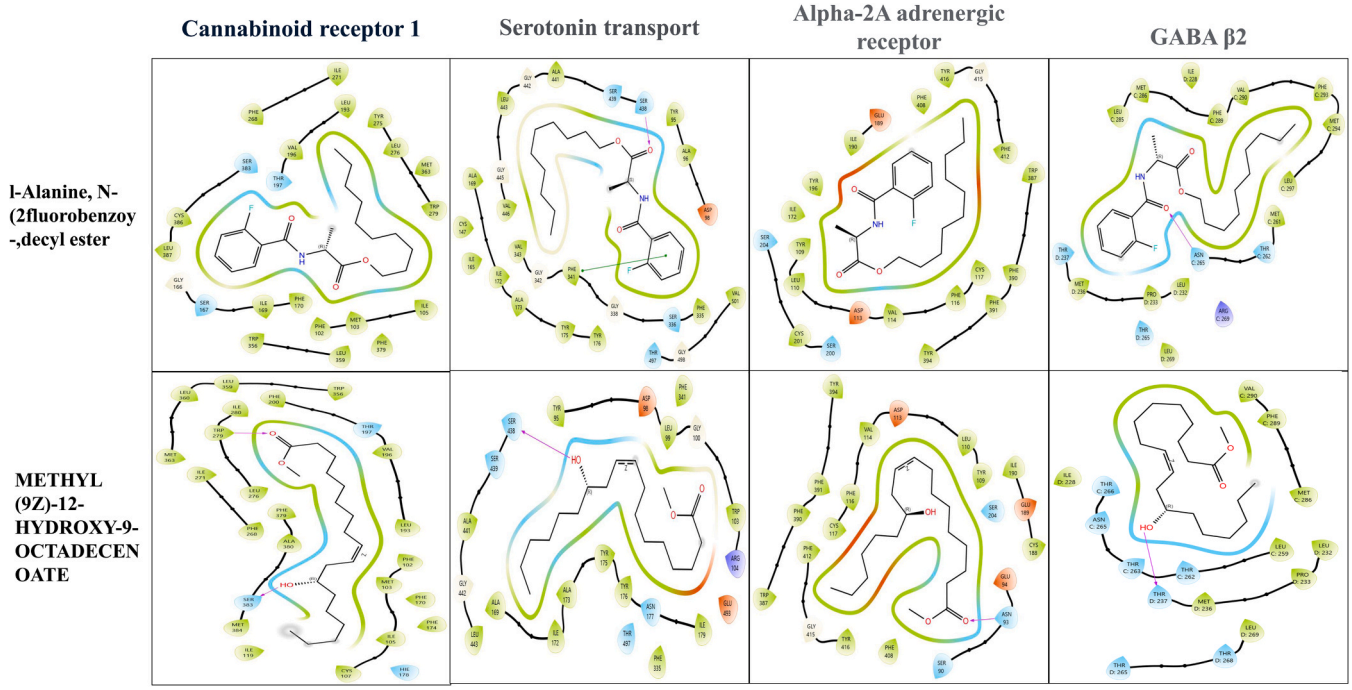

Fig. 6. : The figure represents the molecular interactions between the selected phytoconstituents (l-Alanine, N-(2-fluorobenzoyl), decyl ester, and methyl (9*Z*)-12-hydroxy-9-octadecenoate) of AqCS extract of stem of *Callistemon viminalis cheel* with CB1, SERT, α2A-AR, and GABAβ2.

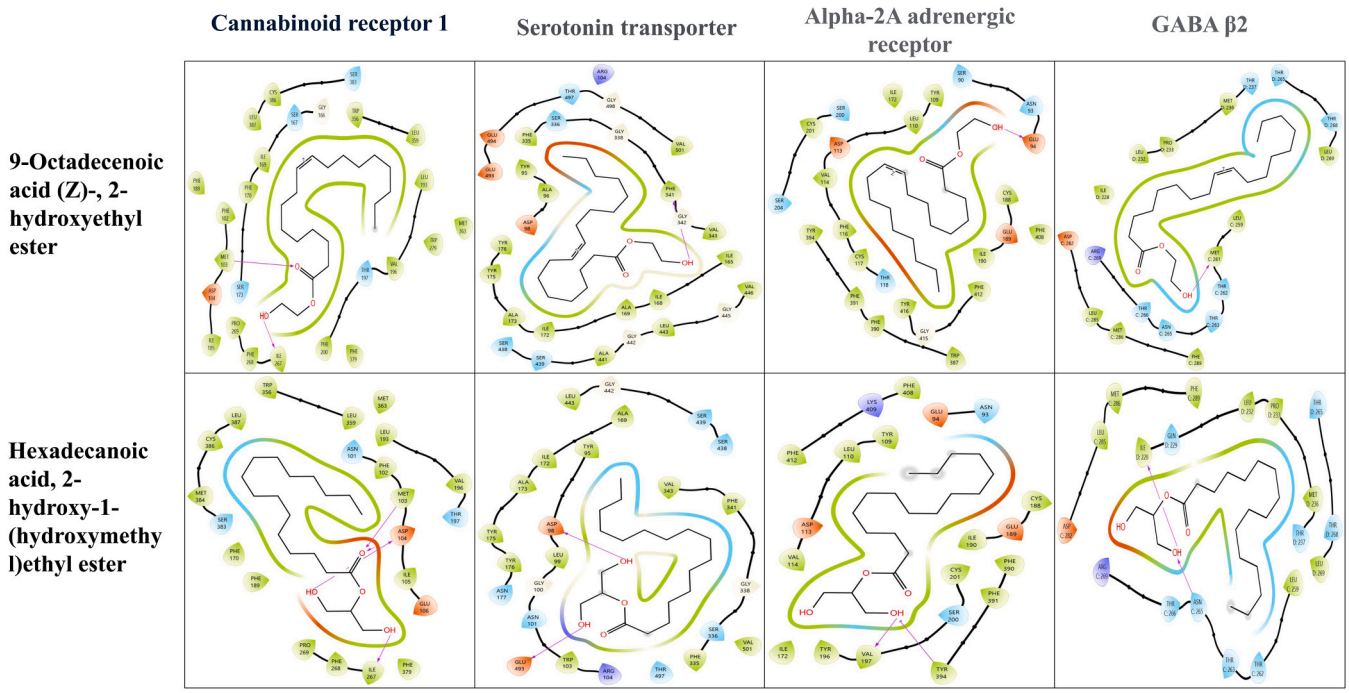

Fig. 7. : The figure characterizes the molecular interactions between the selected phytoconstituents (9-Octadecenoic acid (Z), 2-hydroxyethyl ester, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester) of MeCS extract of stem of *Callistemon viminalis cheel* with CB1, SERT, α2A-AR, and GABAβ2.

molecular weight, lipophilicity (LogP), hydrogen bond donors, and acceptor values (Supplementary Tables 3 and Supplementary Tables 4, respectively). Subsequently, these compounds were utilized as ligands for MD studies.

3.4. Evaluation of MD

The bioactive phytoconstituents selected from the AqCL, MeCL, ChCL, and PtCL extract of the leaf (22, 23, 19, and 20) and AqCS, MeCS, ChCS, and PtCS extract of the stem (24, 29, 11, and 29), respectively complying with Lipinski's Rule, were subjected to interaction studies with an initial target protein, CB1. Docking simulations were performed, and the scores were obtained for the interaction of the bioactive ligands with the initial target protein CB1 (PDB ID: 5TGZ) (Supplementary Tables 5: for stem and Supplementary Tables 6 for leaf). While several bioactive phytoconstituents exhibited interactions with the targeted protein structure (CB1), we narrowed down our focus to the top 10 bioactive phytoconstituents of (AqCL, MeCL, ChCL, and PtCL) and (AqCS, MeCS, ChCS, and PtCS) extracts for both leaf and stem respectively, based on their respective docking scores. The binding affinity between these top 10 bioactive ligands of individual extracts for both leaf and stem with CB1 was characterized by intermolecular hydrogen bonds, indicating their potential as anxiolytic agents. Subsequently, the selected top 10 bioactive phytoconstituents from each extract of the leaf and stem were further examined for their interactions with SERT (PDB ID: 6AWO), α2A-AR (PDB ID: 6KUY), and GABAβ2 (PDB ID: 6X3X), based on MD scores, revealing diverse binding patterns influenced by ligand nature (Supplementary Table 7 for leaf and Supplementary Table 8 for stem).

3.4.1. Screening of promising therapeutic extracts and phytoconstituents

Convincingly, we select the aqueous and methanolic extracts of leaf and stem based on higher docking scores (against CBI, SERT, α2A-AR, and GABAβ2) of the phytoconstituents present in them. These extracts of leaf and stem demonstrate greater docking scores against SERT, α2A-AR, and GABAβ2 rather than the chloroform and petroleum ether extracts (Supplementary Tables 9 for leaf and Supplementary Table 10 for stem). Precisely, the AqCL extract including 9-Octadecenoic acid (*Z*)-2,3-dihydroxypropyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester phytoconstituents (Fig. 4), and the MeCL extract including 9-Octadecenoic acid (*Z*)-2-hydroxyethyl ester, Squalene (Fig. 5) have been considered as a promising herbal compound that has potent interaction with diverse receptor site. Similarly, the AqCS extract comprises l-Alanine, N-(2-fluorobenzoyl), decyl ester, methyl (9*Z*)-12-hydroxy-9-octadecenoate (Fig. 6) and MeCS extract containing 9-Octadecenoic acid (*Z*)-, 2-hydroxyethyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (Fig. 7) phytoconstituents that can be emerged as promising therapeutic agents. These extracts of leaf and stem were selected based on the highest docking scores or protein interaction of phytoconstituents with CB1, α2A-AR, SERT, and GABAβ2 suggesting potential therapeutic effects (Table 5). The intricate interactions of these compounds indicate a substantial influence on pathways associated with neurodegenerative diseases.

3.5. Therapeutic evaluation of preliminary study

An EPM is a widely used apparatus for behavioral studies, particularly involving mice, to evaluate anxiety-like behavior. Parameters such as time spent in the open and closed arms, as well as the number of entries into each arm, are measured to assess anxiety levels in the mice. The results revealed a significant reduction ($F_{10,55} = 84.565$, $p<0.050$) in time spent in the open arms by disease control mice (115.16 ± 8.51 sec) compared to normal control mice (229.16 ± 7.67 sec). In contrast, the total time spent by treated mice, receiving various extracts of the leaf (AqCL, MeCL, ChCL, PtCL) or stem (AqCS, MeCS, PtCS), was significantly increased ($F_{10,55} = 84.565$, $p<0.050$) to (198.5 ± 10.89 sec, 178.16 ± 8.72 sec, 158.33 ± 10.83 sec, 138.33 ± 10.59 sec) for leaf extracts, and (165 ± 9.71 sec, 158.66 ± 9.33 sec, 151.83 ± 10.1 sec) for stem extracts, respectively (Fig. 8). However, treatment with the ChCS extract did not show a desirable effect (129.5 ± 10.44 sec) compared to the disease control. Additionally, the number of entries into the open arms was significantly reduced ($F_{10,55} = 178.52$, $p<0.050$) in disease-control mice (15.5 ± 0.54) compared to normal control mice (24.66 ± 0.51). Conversely, mice treated with different

Table 5
Bioactive compounds of Callistemon viminalis cheel aqueous and methanolic extract of leaf and stem scored the highest docking scores.

Bioactive compounds of aqueous and methanolic extract of leaf					
AqCL					
S.No.	**Compounds**	**Cannabinoid Receptor CB1**	**α2 adrenergic receptor**	**Serotonin transporter**	**Gamma-aminobutyric acid receptor subunit beta−2**
1.	9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester	−13.016	−8.121	−6.268	−7.324
2.	Hexadecanoic acid, 2-hydroxy−1-(hydroxymethyl)ethyl ester	−10.931	−6.881	−6.973	−7.359
MeCL					
1.	9-Octadecenoic acid (*Z*)-, 2-hydroxyethyl ester	−11.911	−5.214	−6.787	−7.5
2.	Squalene	−9.976	−6.345	−6.677	−5.144
Bioactive compounds of aqueous and methanolic extract of stem					
AqCS					
1.	l-Alanine, N-(2-fluorobenzoyl)-, decyl ester	−10.175	−7.144	−6.435	−8.321
2.	METHYL (9*Z*)−12-HYDROXY−9-OCTADECENOATE	−9.492	−7.509	−5.84	−6.124
MeCS					
1.	9-Octadecenoic acid (*Z*)-, 2-hydroxyethyl ester	−12.008	−7.938	−5.214	−7.5
2.	Hexadecanoic acid, 2-hydroxy−1-(hydroxymethyl)ethyl ester	−10.144	−6.881	−6.973	−7.359

extracts of the leaf (AqCL and MeCL) and stem (AqCS, and MeCS) showed significantly more entries into the open arms (21.5 ± 0.54 and 20.33 ± 0.51 for leaf extracts, and 17.66 ± 0.51 and 19.5 ± 0.54 for stem extracts) compared to disease control mice (15.5 ± 0.54) (Fig. 8). Other extracts of the leaf (ChCL and PtCL) and stem (ChCS and PtCS) did not show a significant increase in open-arm entries compared to disease-control animals.

In addition to the EPM test, serum cortisol levels were evaluated, as elevated cortisol is a compelling indicator of anxiety. The results showed a significant increase in cortisol levels ($F_{10,55} = 248.443$, $p<0.050$) in disease-control mice (12.52 ± 0.31 ng/ml) compared to normal control (6.26 ± 0.12 ng/ml). Conversely, significant reductions in cortisol levels ($F_{10,55} = 248.443$, $p<0.050$) were observed in mice treated with various extracts of the leaf (AqCL, MeCL, ChCL, PtCL: 8.38 ± 0.26 ng/ml, 8.68 ± 0.2 ng/ml, 9.52 ± 0.3 ng/ml, 10.36 ± 0.21 ng/ml, respectively) and stem (AqCS, MeCS, ChCS, PtCS: 9.65 ± 0.13 ng/ml, 9.34 ± 0.22 ng/ml, 10.57 ± 0.36 ng/ml, 10.51 ± 0.29 ng/ml, respectively) compared to disease control mice (12.52 ± 0.31 ng/ml). Thus, based on the preliminary therapeutic effects observed, the aqueous and methanolic extracts of both the leaf and stem of the plant exhibit significantly higher potential compared to other extracts (Fig. 8).

4. Discussion

The widespread impact of anxiety on global and daily life underscores the urgent need for effective treatments for prevalent mental ailments. Despite the elusive etiology of anxiety, our investigation into *CVC* reveals promising therapeutic potential. A thorough pharmacognostic evaluation of *CVC*, encompassing organoleptic, morphological, and pharmacognostic parameters, was conducted to establish plant integrity and purity. The analysis included inorganic residue yields, such as total ash, acid-insoluble ash, and water-soluble ash, which indicated the absence of contaminants. Toxicity and safety analyses of different extracts including (AqCL, MeCL, ChCL, and PtCL) from the leaf and (AqCS, MeCS, ChCS, and PtCS) stem of *CVC* showed no adverse effects on vital organs. Toxicity evaluations, both in vivo and in vitro, including LD_{50} values, hemocompatibility, and oxidative parameters, confirmed the non-toxic profile of our botanical specimen. The absence of heavy metals further supported the safety of the plant material. The endorsement of a nontoxic nature was established based on similar observations reported for other natural or synthetic materials in previous studies [14,15,17,18,36]. Phytochemical analysis revealed the presence of flavonoids and phenolics across all extracts, suggesting a wide range of pharmacological activities, including antibacterial, antifungal, antitumoral, allelopathic, hemolytic, antioxidant, antiviral, antiplatelet, anti-inflammatory, antihelminthic, molluscicidal, insecticidal, and hepatoprotective properties as supported by previous reports [6–8,37,38]. GC-MS analysis explored the broad spectrum of phytochemicals in the different extracts of leaf and stem of *CVC* (AqCL, MeCL, ChCL, and PtCL for leaf and AqCS, MeCS, ChCS, and PtCS for stem, respectively). This detailed chemical profiling fills a significant gap in the literature and establishes essential standards for future research.

Screening bioactive phytoconstituents using Schrödinger's Maestro software suite and adhering to "Lipinski's Rule of Five" identified promising compounds. MD studies revealed significant binding affinities of these phytoconstituents with CB1, SERT, α2A-AR, and GABAβ2. Notably, compounds such as 9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester from the AqCL and MeCL extracts, and l-Alanine, N-(2-fluorobenzoyl)-, decyl ester, and methyl (9*Z*)-12-hydroxy-9-octadecenoate from the AqCS and MeCS extracts, exhibited the highest docking scores. These findings suggest that the identified phytochemicals hold significant promise as therapeutic agents, particularly for treating anxiety and neurodegenerative diseases. These promising extracts of leaf and stem were further validated for their therapeutic potential by performing preliminary evaluations in the established mice models of anxiety [39,

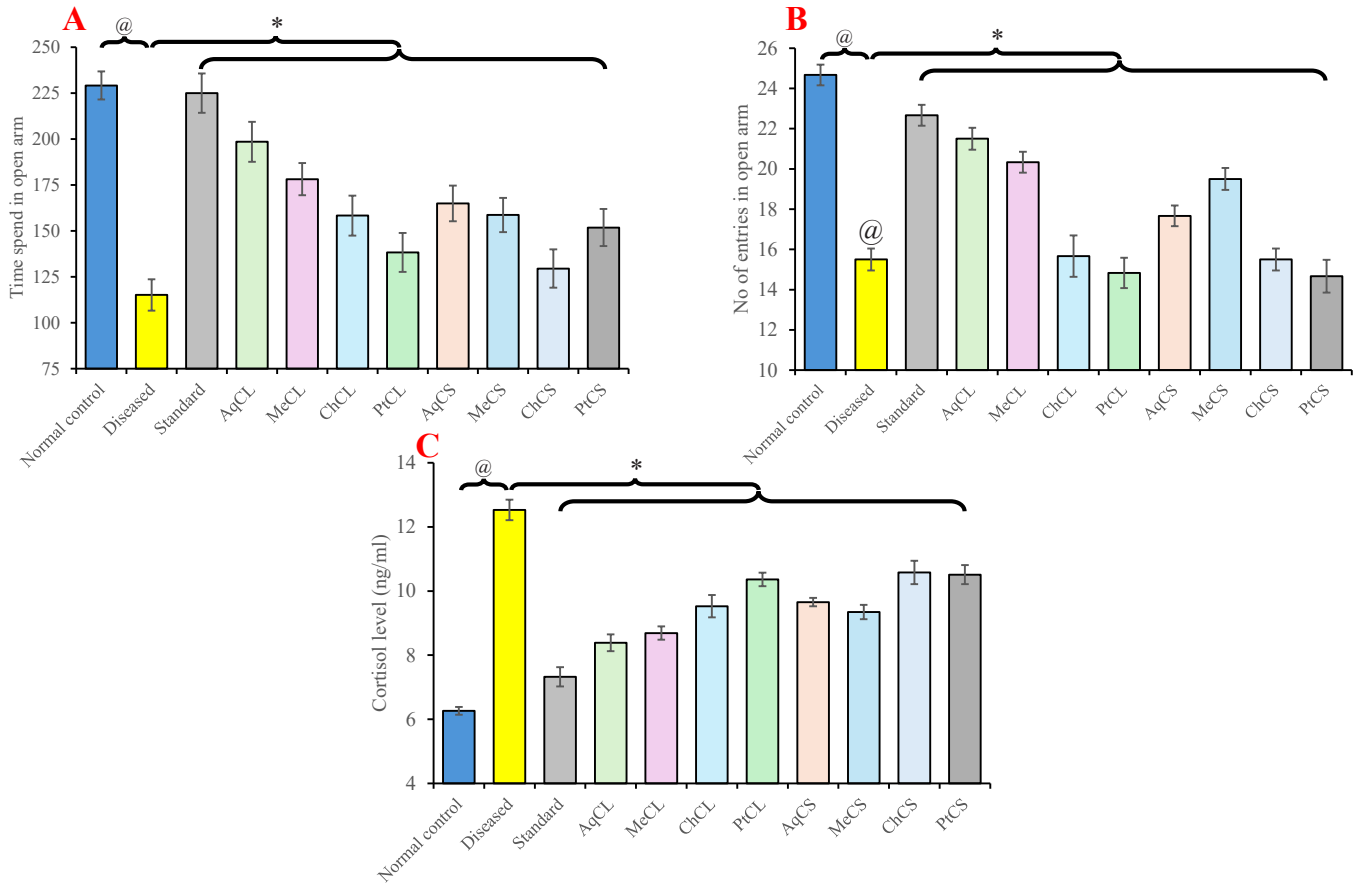

Fig. 8. : The figures represent the effect of different extracts of leaf (AqCL, MeCL, ChCL, and PtCL), and (AqCS, MeCS, ChCS, and PtCS) and stem of *Callistemon viminalis cheel* on time spend in open arm (A) and number of entries in open arm (B) using EPM, and level of serum cortisol level (ng/ml) (C). All values were expressed as mean ± SD whereas, '@' represents $p < 0.05$ vs Normal Control; '*' represents $p < 0.05$ vs disease control group.

40]. The outcomes of EPM revealed the significant anxiolytic effects of the AqCL and MeCL extracts of both leaf and stem as compared to disease control mice and other extracts. The previous reports showed anxiolytic impact supports our outcomes, based on the effect on time spent and the number of entries in the open arm [41,42]. Moreover, the serum cortisol level which is a compelling indicator of anxiety [43], significantly warrants the therapeutic effect of the AqCL and MeCL extracts of both leaf and stem based on significantly reduced cortisol levels. These comprehensive findings provide a solid foundation for future pharmacological research and potential drug development. They emphasize the need for further in vivo and clinical studies to explore the therapeutic potential and safety profiles of these compounds. This study not only demonstrates the significant anxiolytic effects of *CVC* extracts but also highlights their broader pharmacological potential, paving the way for novel therapeutic strategies in treating anxiety and related disorders.

5. Conclusions

The outcomes of our study identify the aqueous and methanolic extracts derived from the leaves and stems of *CVC* as potent therapeutic agents for anxiety. Both extract types were non-toxic and contained numerous unexplored phytoconstituents. The bioactive compounds from these extracts showed significant molecular interactions with CB1, SERT, α2A-AR, and GABAβ2, which are known to be involved in anxiety disorders. Specifically, the AqCL extract contains 9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, while the MeCL extract contains 9-Octadecenoic acid (*Z*)-, 2,3-dihydroxypropyl ester, and Squalene. The AqCS extracts contain l-Alanine, N-(2-fluorobenzoyl), decyl ester, and methyl (9*Z*)-12-hydroxy-9-octadecenoate, and the MeCS extract contains 9-Octadecenoic acid (*Z*)-, 2-hydroxyethyl ester, and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester. These compounds exhibit high potency in addressing anxiety disorders, suggesting their suitability for further research and potential use in anxiety treatments. The phytoconstituents with the highest docking scores, present in specific extracts, were further validated for their therapeutic effects. The results of the preliminary analysis endorsed that these extracts were also therapeutically more effective compared to all other extracts. Thus, our findings highlight the promising role of the leaf and stem of *CVC* extracts (aqueous and methanolic) as potential therapeutic interventions for anxiety disorders, encouraging further exploration and development in this area.

Statements and Declaration

Funding declaration

The author declared no funding was available for the current study.

CRediT authorship contribution statement

Arun K Sharma: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization. **Neelam Kumari:** Writing – review & editing, Writing – original draft, Investigation, Data curation. **Arpana Rana:** Formal analysis. **Arun Mittal:** Validation, Supervision, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No conflict of interest.

Data availability

No data was used for the research described in the article.

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Note

All authors state that there is no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2024.101726.

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