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Research article

Chemical profiles and pharmacological attributes of *Apis cerana indica* beehives using combined experimental and computer-aided studies

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ABSTRACT

The current study sought to determine the anxiolytic, antidepressant, and anti-inflammatory properties of distilled water-soluble extract of beehive (WSE-BH). Gas chromatography–mass spectrometry (GC-MS) studies were used to characterize the chemical compositions obtained from beehives extracted in water and methanol (also fractions). The GC-MS analysis identified 19 compounds in WSE-BH, including high total phenol and flavonoid contents, compared with the methanol extract (21 compounds), ethyl acetate fraction (9 compounds), and CCl_4 fraction (27 compounds). The oral administration of WSE-BH (50 and 150 mg/kg) showed significant anxiolytic activities assessed by time spent in (30.80% and 39.47%, respectively) and entry into (47.49% and 55.93%, respectively) the open arms of the elevated plus-maze (EPM). Only the 150 mg/kg dose resulted in a significant effect on the number of head-dipping events in the hole-board test (HBT) (40.2 ± 2.33; $p < 0.01$) vs. diazepam (64.33 ± 3.16; $p < 0.001$). Both the 50 and 150 mg/kg doses resulted in significant ($p < 0.001$) decreases in immobility in the forced swim test (FST) and tail suspensions test (TST), corresponding to the effect of fluoxetine. WSE-BH inhibited histamine-induced paw edema significantly beginning at 60 min, with the 150 mg/kg dose having the highest effect at 180 min. The current findings suggested that WSE-BH had anxiolytic, antidepressant, and anti-inflammatory properties.

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1. Introduction

Recently, both researchers and medical professionals have shown great interest in the potential role played by inflammation in the pathophysiology of mental disorders. Recent studies have demonstrated a strong association between high levels of circulating inflammatory cytokines and depressive and anxiety symptoms [1]. Although psychiatric disorders are historically not considered inflammatory diseases, researchers have proposed that inflammation may represent a significant contributor to the pathogenesis of depression and anxiety disorders [2,3].

Inflammation is generally characterized as an immune response involving the activation of inflammatory cytokines (intracellular and extracellular) that mediate cell damage. The inflammatory pathway is typically induced to counteract invading pathogens but can also cause self-damage, as in the case of self-immunity [4]. Chronic autoimmune inflammation plays an important role in the pathophysiology of chronic inflammatory diseases, such as asthma, cancer, cardiovascular diseases, and arthritis [1,5–7]. Histamine is an important inflammatory mediator involved in the allergy response, which is often associated with acute inflammation activated by the histamine H_1 receptor (H_1R) [7]. H_1R is primarily expressed in endothelial cells, smooth muscle cells, and the brain, and receptor activation results in vasodilation, increased cellular permeability [8], and an increased pain response [9], in addition to increasing intracellular Ca^{2+} levels and molecular nitric oxide (NO) production [10]. Increasing evidence supports the importance of histamine in anxiety and depression. In several studies, histamine and the stimulation of H_1R or H_2R have been shown to reduce anxiety [11]. H_3R functions as a heteroreceptor that controls the release of several neurotransmitters in the central nervous system (CNS), including GABA, serotonin, and dopamine [12].

The monoamine hypothesis states that the abnormal biotransformation of monoamine oxidases (serotonin, dopamine, and norepinephrine) in the central nervous system causes depression [13]. Several studies have identified oxidative stress as a potential component in the etiology of depression, and the upregulation of reactive oxygen species (ROS) has been observed in the brains and plasma of severely depressed patients [14]. ROS and reactive nitrogen species are known to modulate biogenic amine levels and activities, [15]. Although some antioxidants identified in the body have been shown to neutralize the harmful effects of oxidative stress via various defense mechanisms, during depression, these mechanisms lose their efficacy, resulting in altered pro-inflammatory cytokine (e.g., tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) release and the acceleration of free radical formation [16]. Some anti-inflammatory and antidepressant agents, such as nonsteroidal anti-inflammatory drugs and selective serotonin reuptake inhibitors, exert antidepressant effects by reducing oxidative stress and inflammation [17]. However, these synthetic agents are associated with significant side effects (e.g., daytime drowsiness, elevated blood pressure, reduced sperm concentrations, abnormal sperm DNA fragmentation, and toxicity) [18,19]. Therefore, natural products became attractive to researchers for developing new therapeutic approaches with effective pharmacological targets and fewer side effects due to easy access and reduced costs.

Apis cerana indica, also known as the Indian bee, is an economically valuable bee species in China and has been domesticated in Bangladesh, Pakistan, Thailand, Nepal, India, Myanmar, and Sri Lanka, where they form hives in electrical poles, B-type wooden bee boxes, or tree trunks. Bee products traditionally possess antimicrobial, antioxidant, and anti-inflammatory activities. Honeybees produce honey in honey beehives, which are closely packed hexagonal cells formed of wax that contains essential oils, caffeic acid, flavonoids, isoflavonoids, sinapinic acid, resins, microcomponents, chrysin, isoferulic acid, pollen, and water [20]. In honey beehives, honey is stored in both the lower and upper sections. In general practice, after the honey has been collected, the hives are regarded as waste products, and no use has been identified for the hive material except as wax products; however, the hives contain a diversity of chemicals depending on the nectar sources that may have unprecedented pharmacological efficacy [21]. Previously Tareq et al. (2020) reported the neuropharmacological effects of water-soluble extracts of *Apis cerana indica* beehive (WSE-BH) with different dose (200 and 400 mg/kg) [22]. However, there are no study for *in vitro* antioxidant, thrombolytic, and cytotoxic activities using WSE-BH. As well, the *in vivo* anxiolytic, antidepressant and anti-inflammatory study performed in lower dose. Therefore, the current goals to explore the pharmacological properties of WSE-BH and identify the potential bioactive compounds that mediate these effects using GC-MS. In addition, a computational analysis using molecular docking and analysis of absorption, distribution, metabolism, excretion, and toxicity (ADME/T) was performed to understand the compounds' selected activities.

2. Materials and methods

2.1. Chemicals

DPPH were bought from Sigma-Aldrich (St. Louis, USA). Fluoxetine hydrochloride and diazepam were acquired from Square Pharmaceutical Ltd. (Dhaka, Bangladesh). Tween-80 was attained from Scharlab (Sentmenat, Barcelona, Spain). Folin–Ciocalteu reagent (FCR), gallic acid, isopropyl alcohol, quercetin was procured from Merck (Darmstadt, Germany). All other chemicals were analytical grade and purchased from local provider.

2.2. Extraction and Solvent–Solvent partitioning

The details regarding the beehive collection and extraction procedures for WSE-BH were described in detail in an earlier study [22]. This study represents a continuation of the study of the previously prepared WSE-BH, which was preserved at 4 °C in a Falcon® tube. Similarly, a crude methanolic extract was prepared and subjected for solvent–solvent partitioning, according to the Kupchan and Tsou protocol, using ethyl acetate and carbon tetrachloride (CCl_4) [23,24].

2.3. GC-MS analysis and bioactive compound identification

The GC-MS analysis of WSE-BH, methanolic extract, and the ethyl acetate and CCl_4 fractions were assessed as previously described process of Mazumder et al., 2020 [25,26].

2.4. Total phenolic content (TPC)

The TPC of WSE-BH was quantified using FCR as the oxidizing agent. During this method, polyphenolic contents in the test sample display a blue color after being reduced by FCR. FCR (1 mL) was diluted in distilled water (9 mL), and the diluted FCR (2.5 mL) was mixed with 20% Na_2CO_3 (2.5 mL) and 500 μg/mL extract. After incubating the solution at 25 °C for 20 min, the absorbance was measured and recorded at 765 nm with the UV spectrophotometer. The absorbance was measured in triplicate. For the TPC determination, gallic acid was utilized to generate a standard curve, and TPC values were represented in milligrams of gallic acid equivalents (GAE) per gram. Therefore, the same FCR reduction protocol was applied for known quantities of gallic acid, and a standard calibration curve was attained by plotting concentration vs. absorbance [27].

2.5. Total flavonoid content (TFC)

The TFC of the extract was estimated using a standard colorimetric method. WSE-BH (0.5 mL) was combined with methanol (1.5 mL), 1 M CH_3CO_2K (0.1 mL), 10% $AlCl_3$ (0.1 mL), and distilled water (2.8 mL). The mixture was incubated at 25 °C for 30 min, and the absorbance was taken in triplicate manner at 415 nm. For this experiment, quercetin was used to generate a standard curve, and the TFC was expressed as milligrams of quercetin equivalents (QE) per gram [28].

2.6. DPPH radical scavenging activity

The DPPH radical scavenging activity of WSE-BH was determined using the method reported by Braca et al. (2001) [29]. DPPH solution (4 mg DPPH in 100 mL 95% methanol) was added to test tubes containing WSE-BH and ascorbic acid, respectively with serially-diluted concentrations of 15.12–500 μg/mL each, while the mixed solution was incubated for 30 min at 25 °C. The absorbance at 517 nm was measured using a UV spectrophotometer, and the percentage of radical scavenging activity was determined as follows (Equation (1)):

$$\%\ Scavenging\ activity = \frac{(absorbance\ of\ control - absorbance\ ofextract\ or\ standard)}{absorbance\ of\ control} \times 100 \quad (1)$$

2.7. Thrombolytic activity

Formerly, donor volunteers received a consent form containing the research title and purpose accord with investigators' names and contact details. In-depth information including donor's inclusion criteria (non-smokers, non-drinker, no history of using cardiovascular medication), the volume of blood will be needed, estimated time requires for blood sampling, conceivable discomfort at puncture site was explained in the form. The consent form consists of a Yes/No question format with the donor's signature and date.

In vitro clot lysis assay was carried out by described technique Tareq et al., 2020 [27]. BMI, blood pressure (mmHg), heart rate (bpm), and temperature (°C) were used to assess the health of seven male volunteers (aged 22–25 years). Each volunteer had 3 mL of venous blood taken and placed in a pre-weighed eppendorf tube (0.5 mL). The clot was formed after 45 min of incubation at 37 °C in each tube. Clot weight was calculated by reweighing each tube after the discharged serum had been carefully removed without disrupting the clot. Each tube received 100 μL of WSE-BH at a concentration of 5 mg/mL, 10 mg/mL, and 20 mg/mL. In addition, 100 μL of normal saline (the negative control) and streptokinase (the standard) were utilized in experimental observations separately. This was followed by another 90 min of incubation at (37 °C), during which the discharged fluid was carefully separated and weighed once again. The percentage of clot lysis was determined using equation (2).

$$\%\ \text{of clot lysis} = \frac{\text{weight of clot after removing of fluid}}{\text{clot weight}} \times 100 \quad (2)$$

2.8. Brine shrimp lethality bioassay

The *Artemia salina* was utilized to test the cytotoxicity of a WSE-BH. Brine shrimp cysts (2.5 g) were put to a beaker (1 L) of artificial seawater (3.8% NaCl solution/L, w/v) while the temperature and pH were maintained at 25 ± 1 °C and 8.0, respectively. The light intensity for this study was 60 W. The brine shrimp beaker was 9 cm apart from the light. For the shrimp to mature, the shrimp eggs were hatched for 36 h. To make a stock solution with a 5000 μg/mL concentration (50 mg in 5 mL solution), the extract was diluted in 0.9% NaCl. Freshly made artificial seawater (3.8% NaCl solution/L, w/v) was used to get serially diluted concentrations of 31.25–1000 μg/mL. Finally, 10 nauplii were carefully transferred to each test tube, each containing a total volume of 5 mL. Vincristine Sulfate was employed in a concentration of 3.13, 6.25, 12.5, 25 50, and 100 μg/mL to serve as a positive control for the prior procedure. The research was carried out three times. A magnifying lens was used to view all vials after 24 h, and the number of living

nauplii in each vial was recorded [30,31]. The percentage of mortality rate was calculated using equation (3).

$$\% \text{ of mortality} = (N_1/N_0) \times 100 \quad (3)$$

Where N_0 = the number of nauplii taken; N_1 = the number of nauplii dead.

2.9. Behavioral paradigm

This study used Swiss albino mice from the Department of Pharmacy, International Islamic University Chittagong, which was 6–7 weeks old and weighed 25–35 g. During their adaptation period of 14 days, the animals were exposed to 25 ± 2 °C, a 12-h light/dark cycle, freshwater, and food pellets were provided. We conducted all behavioral trials from 10 a.m. to 3 p.m. and monitored the animals for an hour after each experiment was completed. The institutional animal ethical committee approved this work (Pharm/p&d/158/14–20).

2.10. Treatment design

The animals were separated into four groups at random, with each group consisting of five mice: a control group, a reference drug group, and two test groups (WSE-BH: 50 and 150 mg/kg, orally). Fluoxetine (20 mg/kg b. w., intraperitoneal [IP]) was used as the reference drug for antidepressant experiments, diazepam (1 mg/kg b. w., IP) was used as the reference drug for anxiolytic and locomotor tests, and fexofenadine (10 mg/kg b. w., IP) was used as the reference drug during the histamine-induced paw edema model. The control group was treated with 1% Tween-80 in water (10 mL/kg b. w., orally).

2.11. Anxiolytic activity

2.11.1. Hole-board test (HBT)

The HBT is well-known to evaluate anxiety-like actions in rodents. The current experiment was performed in a quiet place, using an apparatus consisting of a board (made of wood; 20 × 40 cm^2) with 16 evenly circulated holes. As mentioned in the treatment design (Section 2.10), the mice were put in the center of the device 30 min after treatment administration. This experiment evaluates the head-dipping activity of the mice, which can be used as a measure of anxiolytic behavior and exploratory activity [32]. A tally counter was used to keep track of how many times the mice dipped their heads into the holes during a 5-min trial period.

2.11.2. Elevated plus-maze (EPM)

The EPM is a well-established test used to determine anxiety in rodents. The EPM, two open arms (35 × 5 cm^2) and two closed arms (35 × 20 cm^2), with a central square (5 × 5 cm^2), were elevated 25 cm above the floor in a dimly lit room. The mice were grouped and treated following the treatment strategy (Section 2.10). During 5 min, the time spent in each arm was tracked and recorded. For the percentage of time spent and the number of entries in the open arms, we used these formulae (Equations (4) and (5)) [33]:

$$(\%)\ \textit{Time spent in open arm} = \frac{\textit{Time spent in open arm}}{\textit{Time spent in open arm} + \textit{Time spent in closed arm}} \quad (4)$$

$$(\%)\ \textit{Entry into open arm} = \frac{\textit{Entry into open arm}}{\textit{Entry into open arm} + \textit{Entry into closed arm}} \quad (5)$$

2.12. Antidepressant activity

2.12.1. Forced swim test

The antidepressant activity of WSE-BH was assessed using a previously published protocol [34]. They were grouped and treated in accordance with the treatment strategy (Section 2.10). Each mouse was placed in a glass apparatus (25 × 15 × 25 cm^3) filled with water (25 ± 2 °C) up to 15 cm, starting 60 min after treatment, and forced to swim for 6 min. The first 2 min of the experiment was regarded as an adjustment period, and the remaining 4 min were evaluated for immobility. The percentage of immobility during the last 4 min was calculated as described in Rahman et al. (2020) [19].

2.12.2. Tail suspension test (TST)

The TST was carried out by hanging mice by their tails with the adhesive tape placed roughly 1 cm from the tip of the tail, beginning 60 min after treatment, as indicated in the treatment design (Section 2.10). The evaluation and calculation of percentage immobility time were calculated as described for the FST method for 6 min observation [19].

2.13. Histamine-induced paw edema

Orally administered WSE-BH was tested for anti-inflammatory properties by injecting histamine into the right paw's sub-plantar area, as described in Yong et al. (2013) [7]. In brief, the mice were grouped and treated as described in the treatment design section for five consecutive days, after which 0.1 mL histamine (0.1%) was injected into the right paw. Changes in the paw thickness, as

measured using a thread, were used to measure edema before and 60, 120, 180, and 240 min after histamine injection.

2.14. Computer-aided drug designing (CADD)

2.14.1. Molecular docking

The identified compounds from WSE-BH were subjected to molecular docking using Schrödinger Maestro (v11.1) as previously described [27,35]. The identified compounds were assessed for docking interaction with selected enzymes/receptor which are responsible for anti-oxidant (PDB: 1R4U) [36], thrombolytic (PDB: 1A5H) [37], anxiolytic (PDB: 4UUJ) [38], anti-depressant (PDB ID: 5I6X) [39], anti-inflammatory (PDB: 2OYE and PDB: 6COX) [40,41] activities. Protein Dara Bank provided the three-dimensional structures of receptors and enzymes [42].

2.14.2. ADME/T

QikProp (Schrödinger v11.1) was used to examine the compounds' drug likeliness profile using Lipinski's rules of five, while admetSAR (Online tool) was used to determine the compounds' toxicological characteristics [43].

2.15. Statistical analysis

The results were reported in the form of mean ± SEM. Statistical analysis, including ANOVA (Dunnett's test), was carried out using GraphPad Prism (version 8.4.2).*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ are the levels of statistical significance.

3. Results

3.1. GC-MS analysis

As indicated in Table 1 and Fig. 1, GC-MS analysis detected 19 compounds in WSE-BH with retention times ranging from 3.764 min to 28.276 min. The major identified compounds were ephedrine (19.43%); clofexamide (19.87%). and silane, dimethyl (3-methylbut-3-enyloxy)isobutoxy- (10.53%). From the methanolic extract, 21 compounds with different retention times were identified, presented in Table 2 and Fig. 2. The major compounds were 2H-pyran-2-one, 5,6-dihydro-6-pentyl- (7.42%); tetradecanoic acid (7.27%); hexadecanoic acid, ethyl ester (7.36%); hexadecanoic acid, 15-methyl-, methyl ester (7.29%); 13-octadecenoic acid, methyl ester (7.14%); methyl stearate (7.29%); hentriacontane (7.18%); 9,12,15-octadecatrienoic acid, (z,z,z)- (7.31%); methyl 13-eicosenoate (0.58%); and 11-eicosenoic acid, methyl ester (7.14%).

A total of nine (9) compounds were identified from the ethyl acetate fraction, with recorded retention times between 3.704 min and 30.809 min. The major compounds, based on the maximum area, were ethylbenzene (18.41%); fenproporex (18.51%); octane, 1,1′-oxybis-(17.91%); and tetradecanoic acid, 12-methyl-, methyl ester, (s)- (18.20%). Information regarding this fraction can be found in Table 3 and Fig. 3.

The CCl_4 fraction was associated with 27 distinct compounds, including the following major compounds: 2(3H)-furanone, dihydro-3-hydroxy-4,4-dimethyl-, (.+/−.)- (8.59%); 2,6,6-trimethyl-2-cyclohexene-1,4-dione (8.56%); decane, 2-cyclohexyl- (8.68%); 2H-pyran-2-one, 5,6-dihydro-6-pentyl- (8.78%); 2-cyclohexen-1-one, 3,5-dimethyl- (8.68%); tetradecanoic acid (8.60%); and 12,15-octadecadienoic acid, methyl ester (8.55%). The GC-MS data for this fraction is presented in Table 4 and Fig. 4.

Table 1
Quantitative compounds identified from water-soluble extract of *Apis cerana indica* beehive by GC-MS analysis.

No	Compounds	Formula	RT	Peak area (%)
1	Ephedrine	$C_{16}H_{31}NOSi_2$	3.764	19.43
2	1-butyl (dimethyl)silyloxypropane	C_9H_{22}	5.882	7.57
3	1-propanamine, 3-(methylthio)-	$C_4H_{11}NS$	6.458	0.10
4	1,3-Dichlorobutane	$C_4H_8Cl_2$	6.974	1.63
5	2-mercaptopropanoic acid	$C_3H_6O_2S$	7.075	2.44
6	Sydnone, 3-neopentyl-	$C_7H_{13}N_2O_2^+$	9.159	3.03
7	Alpha-terpineol	$C_{10}H_{18}O$	11.418	2.36
8	1-dimethyl (prop-2-enyl)silyloxy-10-undecene	$C_{16}H_{32}OSi$	13.523	3.50
9	Silane, dimethyl (3-methylbut-3-enyloxy)propoxy-	$C_{10}H_{22}O_2Si$	13.663	10.53
10	10-(tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol	$C_{15}H_{24}O_3$	14.957	4.29
11	Clofexamide	$C_{14}H_{21}ClN_2O_2$	15.252	19.87
12	Acetic acid, (dodecahydro-7-hydroxy-1,4 b,8,8-tetramethyl-10-oxo-2 (1 h)	$C_{24}H_{39}NO_4$	16.995	2.21
13	Chloroacetic acid, dodec-9-ynyl ester	$C_{14}H_{23}ClO_2$	17.605	2.61
14	*P*-mentha-1,8-dien-7-ol	$C_{10}H_{16}O$	17.678	2.62
15	Methyl 11,12-tetradecadienoate	$C_{15}H_{26}O_2$	19.89	2.61
16	6-Propyl-5,6-dihydro-2H-pyran-2-one	$C_8H_{12}O_2$	20.775	2.63
17	Diethylmalonic acid, monochloride, tetrahydrofurfuryl ester	$C_{12}H_{19}ClO_4$	21.043	3.00
18	Carbonic acid, but-3-yn-1-yl hexadecyl ester	$C_{21}H_{38}O_3$	26.901	7.16
19	Sedoheptulosan	$C_7H_{12}O_6$	28.276	2.41

Here, RT: retention time.

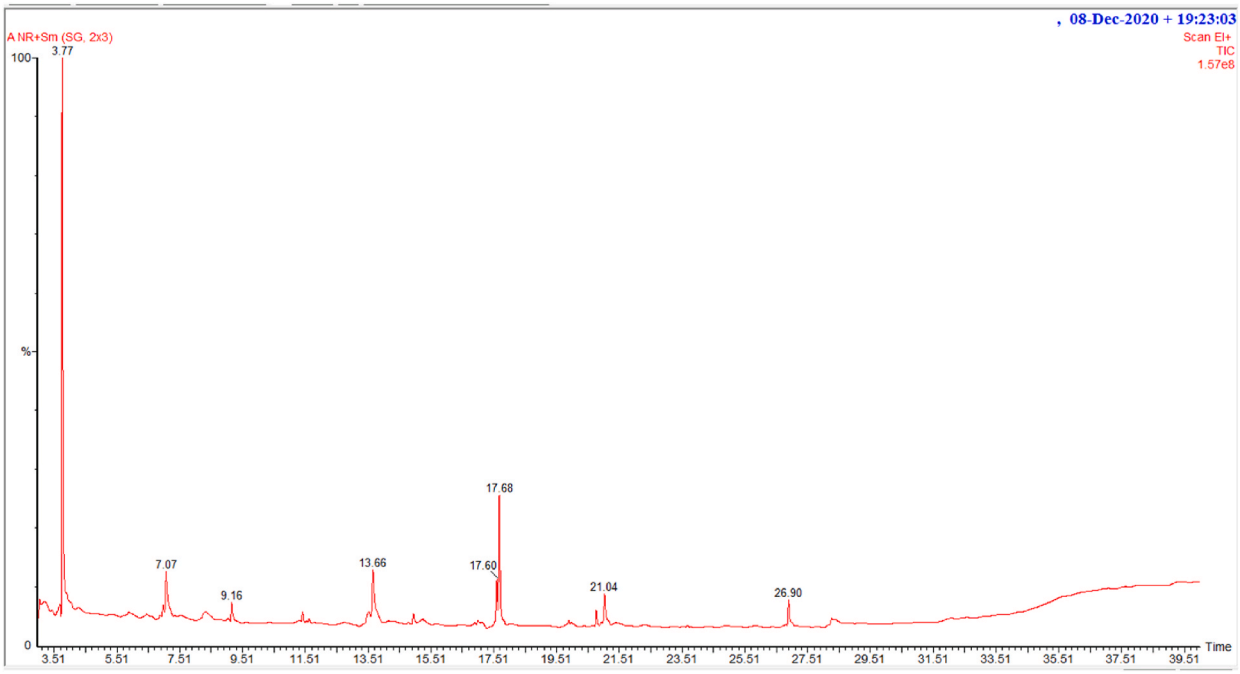

Fig. 1. Major chemical compounds identified in the water-soluble extract of beehive.

Table 2
Quantitative compounds identified from methanol extract of *Apis cerana indica* beehive by GC-MS analysis.

No.	Compounds	Formula	RT	Peak area (%)
1	Boronic acid, ethyl-, bis(2-mercaptoethyl ester)	$C_6H_{15}BO_2S_2$	7.169	0.90
2	*Trans*-linalool oxide (furanoid)	$C_{10}H_{18}O_2$	7.799	0.87
3	2 h-pyran-2-one, 5,6-dihydro-6-pentyl-	$C_{10}H_{16}O_2$	20.773	7.42
4	*N*-ethyl-2-amino-octadec-4-en-1,3-diol methaneboronate	$C_{21}H_{42}BNO_2$	21.028	1.12
5	Decan-2,4,8-triene, 4,7-dimethyl-	$C_{12}H_{20}$	21.671	1.48
6	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	23.313	7.29
7	Carbonic acid, but-3-yn-1-yl tetradecyl ester	$C_{19}H_{34}O_3$	26.9	2.68
8	Tetradecanoic acid	$C_{14}H_{28}O_2$	28.307	7.27
9	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	28.521	7.36
10	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	29.198	7.29
11	13-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	30.914	7.14
12	Methyl stearate	$C_{19}H_{38}O_2$	31.002	7.29
13	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	31.122	0.95
14	9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)-	$C_{19}H_{32}O_2$	31.531	1.40
15	*N*-propyl 11-octadecenoate	$C_{21}H_{40}O_2$	31.98	0.58
16	(Z)-18-octadec-9-enolide	$C_{18}H_{32}O_2$	32.188	1.48
17	Hentriacontane	$C_{31}H_{64}$	32.476	7.18
18	9,12,15-octadecatrienoic acid, (z,z,z)-	$C_{18}H_{30}O_2$	32.597	7.31
19	Methyl 13-eicosenoate	$C_{21}H_{40}O_2$	34.299	0.58
20	11-eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	34.413	7.14
21	Dotriacontyl pentafluoropropionate	$C_{35}H_{65}F_5O_2$	39.36	0.75

Here, RT: retention time.

3.2. Total phenol and flavonoid contents

A quantitative analysis of potentially relevant antioxidants among the phytochemical content was performed by measuring TPC and TFC, based on regression equations. The results are presented in Figure S1. In WSE-BH, TPC was found to be higher (70.18 ± 3.29 mg GAE/g dry extract) than TFC (46.08 ± 1.69 mg QE/g dry extract).

3.3. DPPH free radical scavenging activity

The DPPH scavenging activity of WSE-BH is depicted in Fig. 5 as a dose–response curve**.** The maximum inhibition percentage calculated for WSE-BH was 61.16% ± 0.58% at 500 μg/mL, whereas ascorbic acid showed 98.33% ± 0.33% inhibition at the same

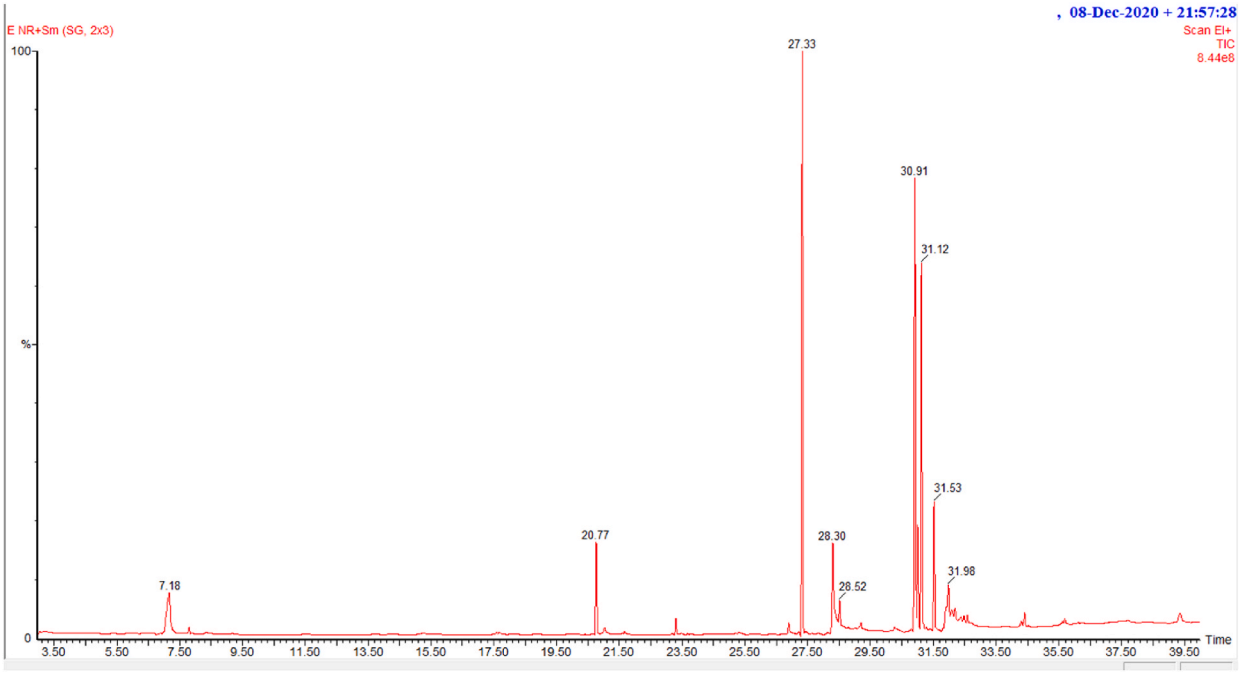

Fig. 2. Major chemical compounds identified in the methanol extract of beehive.

Table 3
Quantitative compounds identified from ethyl acetate extract of *Apis cerana indica* beehive by GC-MS analysis.

No	Compounds	Formula	RT	Peak area (%)
1	Ethylbenzene	C_8H_{10}	3.704	18.41
2	1-(*n*-carbobenzoxyglycyl)-2-benzoylhydrazine	$C_{17}H_{17}N_3O_4$	4.179	4.38
3	Phenylalanine, 4-amino-*n*-t-butyloxycarbonyl-, *t*-butyl ester	$C_{18}H_{28}N_2O_4$	4.24	5.93
4	Propane, 2-fluoro-2-methyl-	C_4H_9F	7.087	2.24
5	*N*-benzyl-*n*-methyl-5,7-dinitro-2,1,3-benzoxadiazol-4-amine	$C_{14}H_{11}N_5O_5$	20.591	4.38
6	Fenproporex	$C_{12}H_{16}N_2$	20.792	18.51
7	Octane, 1,1′-oxybis-	$C_{12}H_{18}OS_4$	21.04	17.91
8	Tetradecanoic acid, 12-methyl-, methyl ester, (s)-	$C_{18}H_{36}O_4$	27.325	18.20
9	Phthalic acid, butyl 2,3-dimethylphenyl ester	$C_{20}H_{22}O_4$	30.809	10.02

Here, RT: retention time.

concentration. The half-maximal inhibitory concentration (IC_{50}) value for WSE-BH (293.52 μg/mL) indicates that the extract had a moderate effect against DPPH free radicals compared with the standard reference drug ascorbic acid (36.26 μg/mL).

3.4. Clot lysis activity

In the current experiment, WSE-BH showed moderate effects when tested at concentrations of 5, 10, and 20 μg/mL, with the strongest effect observed for 5 μg/mL, with a clot lysis ability of 60.06% ($p < 0.001$; Fig. 6). By contrast, streptokinase (the positive control) showed a clot lysis ability of 72.97%, and the lowest clot lysis ability was exhibited by water (3.33%).

The results were reported in the form of mean ± SEM. ***p < 0.001 are the levels of statistical significance via one-way ANOVA (Dunnett's test).

3.5. Brine shrimp lethality bioassay

To assess the cytotoxicity of WSE-BH, a brine shrimp lethality test was performed. In this assay, the half-maximal lethal dose (LC_{50}) represents the minimum concentration necessary to kill 50% of the brine shrimp. The median lethal dose was calculated using regression curve analysis and the best fit line, as shown in Fig. 7. According to these results, WSE-BH was non-toxic when applied to brine shrimp (LC_{50} = 2143.99 μg/mL) compared with the positive control vincristine sulfate (LC_{50} = 2.78 μg/mL).

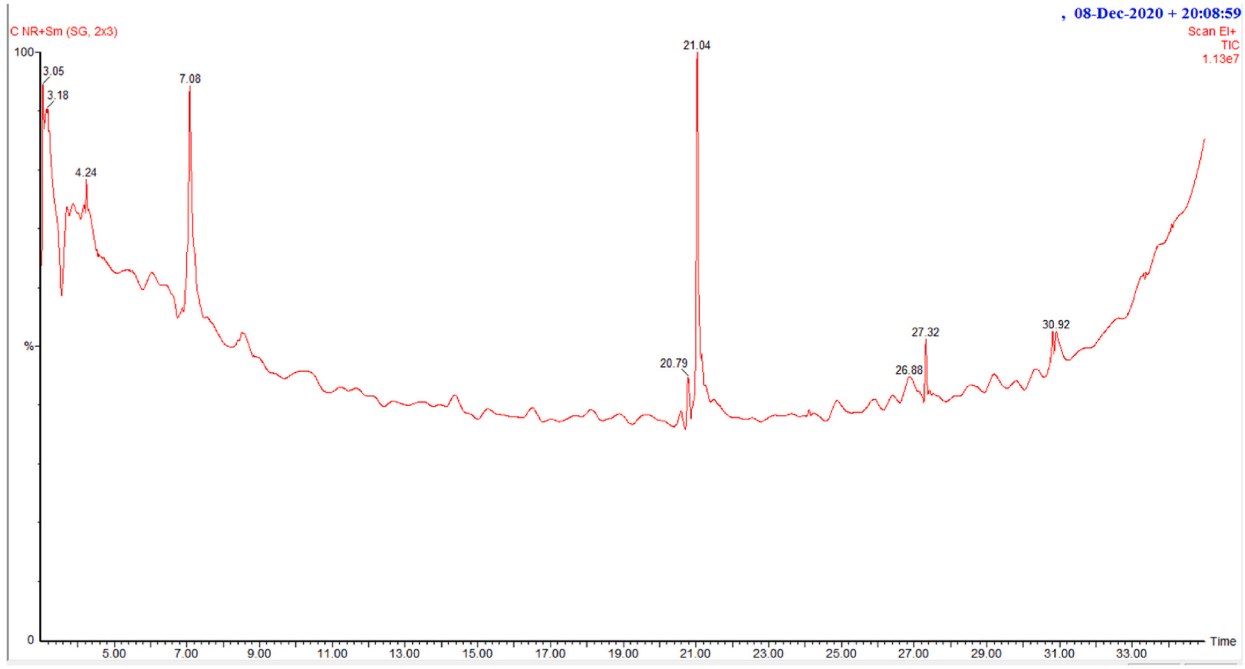

Fig. 3. Major chemical compounds identified in the ethyl acetate fraction of beehive.

Table 4
Quantitative compounds identified from carbon tetrachloride extract of *Apis cerana indica* beehive by GC-MS analysis.

No	Compounds	Formula	RT	Peak area (%)
1	Boronic acid, ethyl-, bis (2-mercaptoethyl ester)	$C_6H_{15}BO_2S_2$	7.201	1.07
2	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	$C_{13}H_{22}O_4$	7.771	1.03
3	*Trans*-linalool oxide (furanoid)	$C_{10}H_{18}O_2$	8.327	1.03
4	2 (3 h)-furanone, dihydro-3-hydroxy-4,4-dimethyl-, (.+/−.)-	$C_6H_{10}O_3$	9.131	8.59
5	Alpha.-terpineol	$C_{10}H_{18}O$	11.396	1.03
6	Cyclopentanecarboxylic acid, cyclopentyl ester	$C_{11}H_{18}O_2$	11.51	1.22
7	2,6,6-trimethyl-2-cyclohexene-1,4-dione	$C_9H_{12}O_2$	11.603	8.56
8	Glutaric acid, 3-methylbut-2-en-1-yl 2-ethylbutyl ester	$C_{16}H_{28}O_4$	12.052	3.16
9	Hexanedioic acid, dimethyl ester	$C_8H_{14}O_4$	13.996	1.04
10	1r,2c,3t,4t-tetramethyl-cyclohexane	$C_{10}H_{20}$	14.11	1.20
11	Decane, 2-cyclohexyl-	$C_{16}H_{32}$	15.162	8.68
12	1-methyl-1-ethoxycyclobutane	$C_7H_{14}O$	15.229	0.98
13	4-amino-2 (1 h)-pyridinone	$C_5H_6N_2O$	15.672	2.98
14	*P*-mentha-1,8-dien-7-ol	$C_{10}H_{16}O$	17.67	1.15
15	Bicyclo [5.1.0]octane, 8-methylene-	C_9H_{14}	17.589	1.67
16	Chloroacetic acid, tetradecyl ester	$C_{16}H_{31}ClO_2$	18.923	1.80
17	2-fluoro-3-trifluoromethylbenzoic acid, 5-pentadecyl ester	$C_{23}H_{34}F_4O_2$	19.795	6.08
18	Phenylacetic acid, dodec-9-ynyl ester	$C_{20}H_{28}O_2$	19.875	2.05
19	2 h-pyran-2-one, 5,6-dihydro-6-pentyl-	$C_{10}H_{16}O_2$	20.753	8.78
20	2-cyclohexen-1-one, 3,5-dimethyl-	$C_8H_{12}O$	21.652	8.68
21	Chromium, tetracarbonylbis (eta.2-ethene)-	$C_8H_8CrO_4$	23.67	0.62
22	6-nonyl-5,6-dihydro-2h-pyran-2-one	$C_{14}H_{24}O_2$	25.292	2.39
23	Glutaric acid, cyclohexylmethyl dec-9-enyl ester	$C_{22}H_{38}O_4$	26.887	3.18
24	Heptacosanoic acid, 25-methyl-, methyl ester	$C_{29}H_{58}O_2$	27.303	1.52
25	Tetradecanoic acid	$C_{14}H_{28}O_2$	28.262	8.60
26	12,15-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	31.105	8.55
27	Methyl 11-cyclohexylundecanoate	$C_{18}H_{34}O_2$	32.366	0.70

Here, RT: retention time.

3.6. Anxiolytic activity

3.6.1. Hole-board test

As shown in Fig. 8, the mice in the positive control (diazepam) group revealed an increased number of head-dipping events (64.33 ± 3.16) compared with the negative control group (26.33 ± 0.88). Treatment with WSE-BH at the 150 mg/kg dose demonstrated a

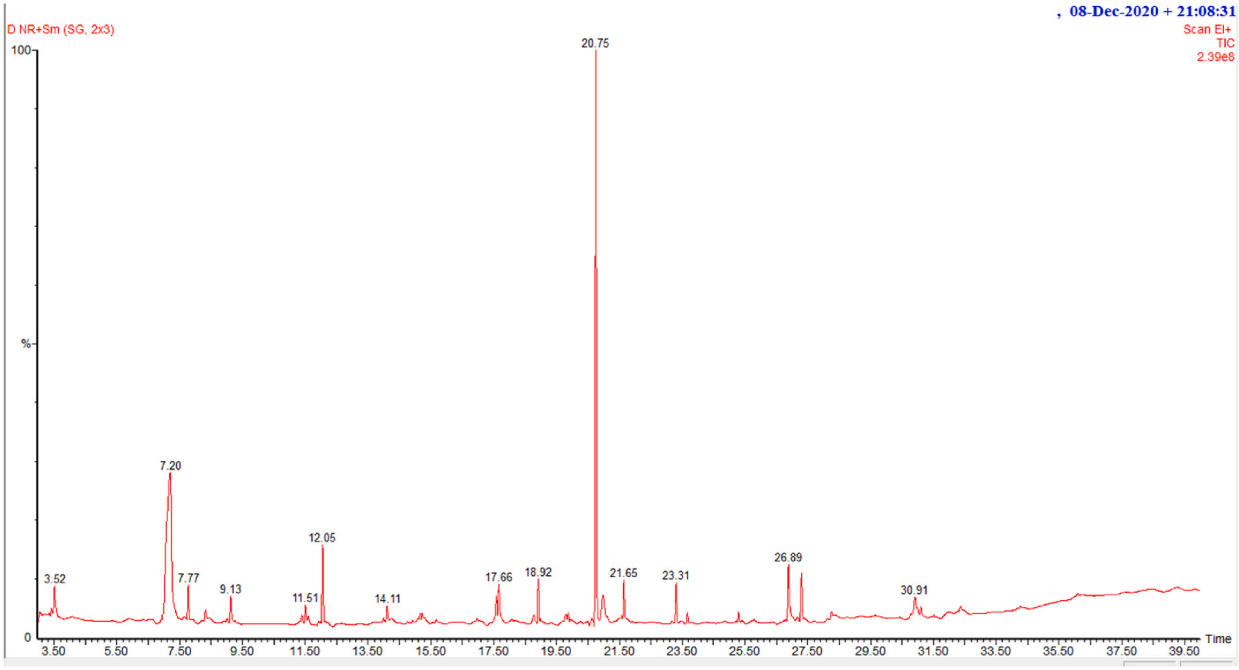

Fig. 4. Major chemical compounds identified in the CCl_4 fraction of beehive.

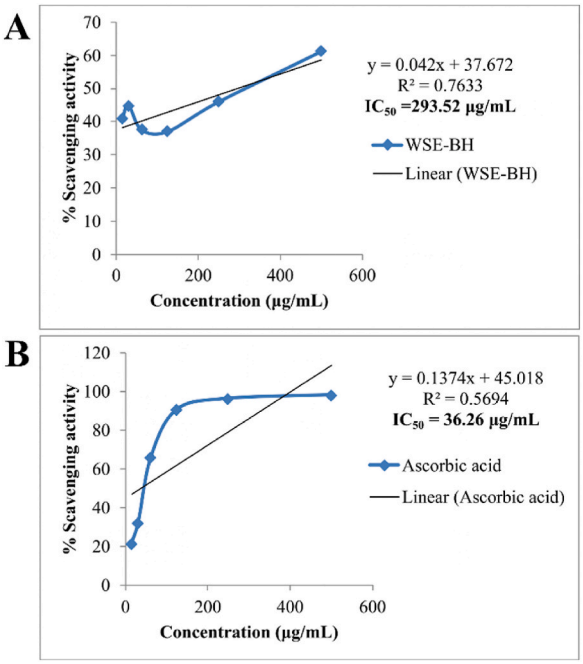

Fig. 5. DPPH scavenging activity of WSE-BH (A) and ascorbic acid (B).

significant increase ($p < 0.01$) in the number of head-dipping events (40.2 ± 2.33) compared with the control group, whereas no significant difference was noticed between the control group and the 50 mg/kg dose of WSE-BH.

3.6.2. Elevated plus-maze

After treatment with WSE-BH (150 mg/kg), mice spent a significantly increased amount of time in open arms of the EPM (39.47% ± 2.58%; $p < 0.01$) compared with the control group, whereas mice treated with 50 mg/kg exhibited spent 30.48% ± 1.06% of the

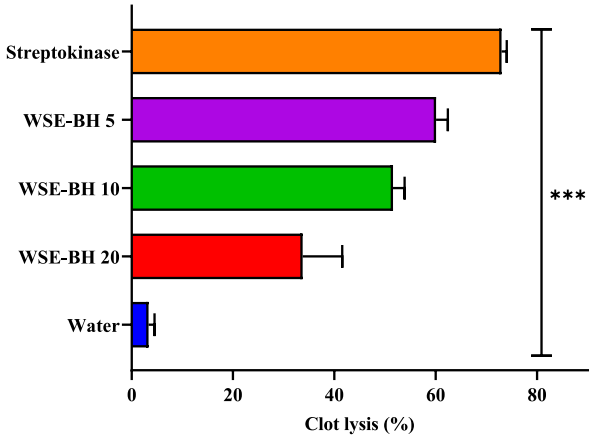

Fig. 6. The clot lysis activity of WSE-BH, streptokinase (SK) and normal saline (control).

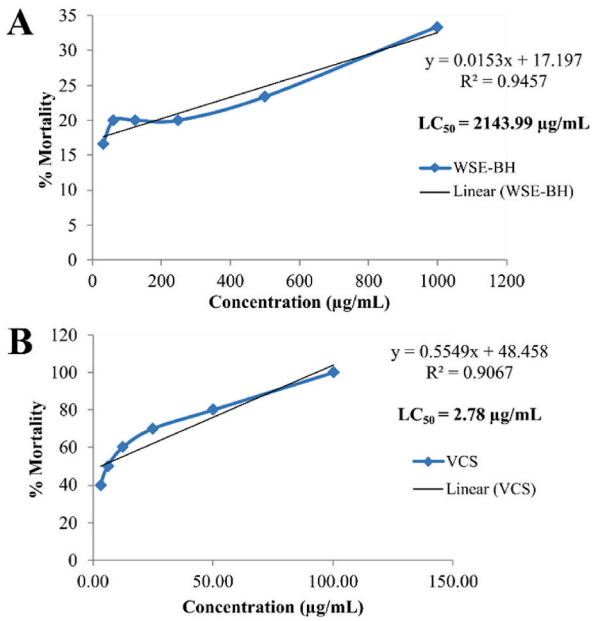

Fig. 7. Cytotoxicity of (A) Water-soluble extract of *Apis cerana* indica beehive (WSE-BH) and (B) Vincristine sulfate (VS) in brine shrimp lethality assay.

time in the open arms, and mice treated with the positive control (diazepam) spent 69.33% ± 1.15% in the open arms ($p < 0.001$). Correspondingly, treatment with WSE-BH at the 150 mg/kg dose increased the proportion of entries into the open arm (55.93% ± 3.43%; $p < 0.01$), as did the 50 mg/kg dose (47.49% ± 1.08%). The positive control unveiled a significant increase in the proportion of open arm entries (66.67% ± 9.27%; $p < 0.001$) in comparison to the control group (28.33% ± 6.01%). The results are shown in Fig. 9 (A, B).

3.7. Antidepressant activity

3.7.1. Forced swim test

Treatment with WSE-BH at both doses (50 mg/kg and 150 mg/kg) demonestrated a significant increase in the antidepressant effect in mice, as assessed by the FST (49.66% and 42.05%, respectively, $p < 0.001$), in comparison to the control group. In addition, the positive control fluoxetine (20 mg/kg) also demonstrated a significant ($p < 0.001$) percentage of immobility (54.45%; Fig. 10A).

3.7.2. Tail suspension test

With the aid of the TST model, the antidepressant properties of WSE-BH were analyzed. As depicted in Fig. 10B, both doses of WSE-BH resulted in a significant change in the immobility time ($p < 0.001$), whereas mice treated with the positive control fluoxetine (20

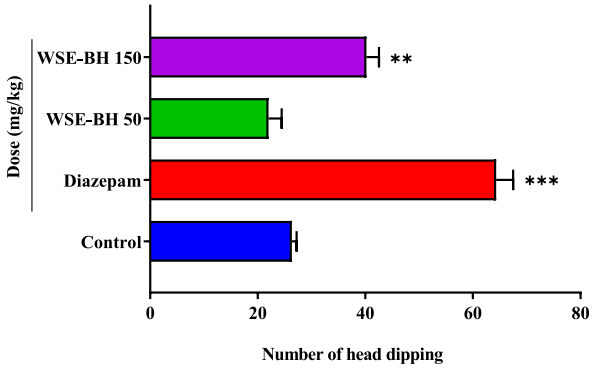

Fig. 8. Anxiolytic activity of WSE-BH and diazepam on number of head-dipping in hole-board test. The results were reported in the form of mean ± SEM. **p < 0.01, and ***p < 0.001 are the levels of statistical significance via one-way ANOVA (Dunnett's test).

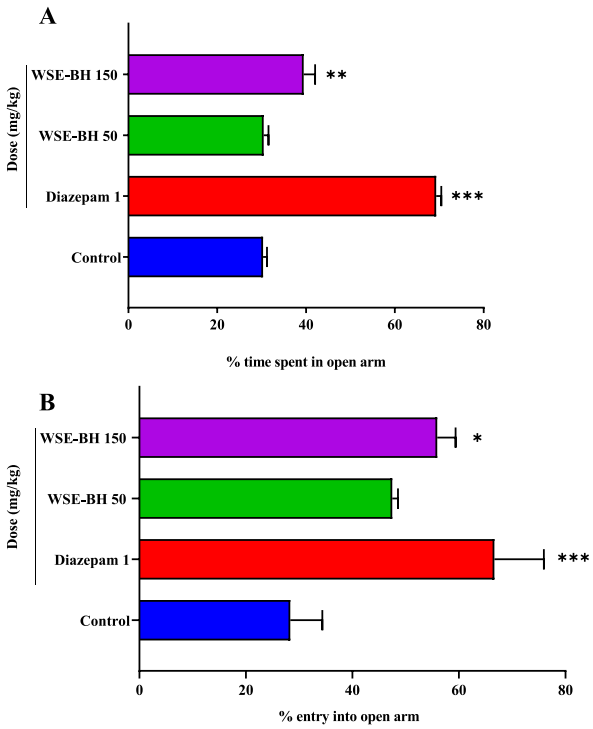

Fig. 9. (A) Anxiolytic activity of Water-soluble extract of *Apis cerana* indica beehive (WSE-BH) and diazepam on % of time spend in open arm in elevated plus maze test. (B) Effect of WSE-BH and diazepam on % of entry in open arm in elevated plus maze test. The results were reported in the form of mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 are the levels of statistical significance via one-way ANOVA (Dunnett's test).

mg/kg) were immobile for only 55.73% (82.33 ± 1.19 s; $p < 0.001$) of the time spent immobile by the control group.

3.8. Anti-inflammatory activity by histamine-induced paw edema

The overall data demonstrated that the various pretreatment groups of mice were able to successfully treat paw edema for up to 240 min following the histamine injection. Significant ($p < 0.001$) effects for edema reduction in comparison to the control group were observed starting as early as 60 min after treatment with both doses of WSE-BH. After 240 min of treatment, the WSE-BH 150 mg/kg dose showed an inhibition of 12.8 ± 0.37 ($p < 0.01$), whereas the 50 mg/kg dose showed an inhibition of 13 ± 0.45 ($p < 0.5$). The positive control fexofenadine showed significant edema reducing effects ($p < 0.001$) when measured at 60 min, 180 min, and 240 min following histamine injection (Figs. 11 and 12).

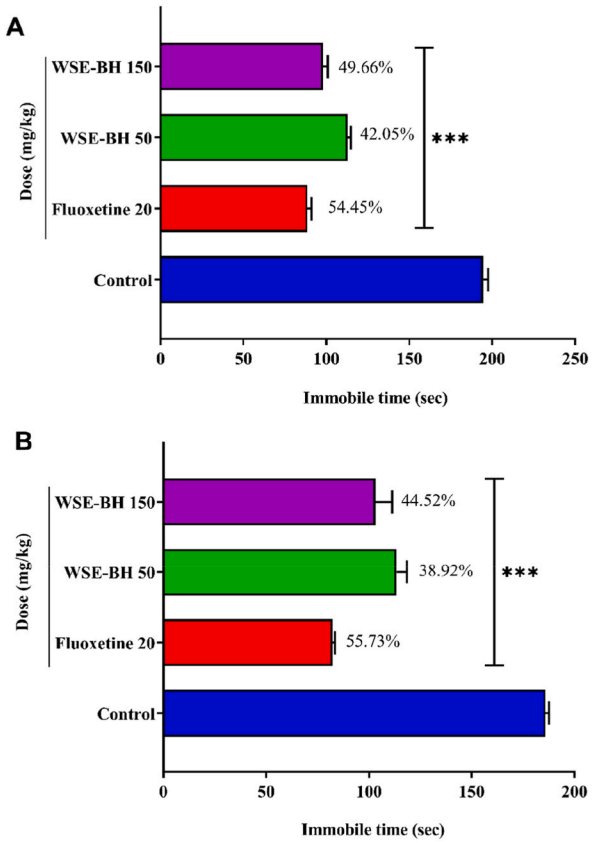

Fig. 10. (A) The activity of WSE-BH and standard drug (fluoxetine) on the force swimming test of mice. (B) The activity of WSE-BH and standard drug (fluoxetine) on the tail suspension test of mice. The results were reported in the form of mean ± SEM. ***$p < 0.001$ are the levels of statistical significance via one-way ANOVA (Dunnett's test).

3.9. Computer-aided drug designing (CADD)

3.9.1. Molecular docking

3.9.1.1. Molecular docking for antioxidant. Table 5 represents the antioxidant molecular docking study of sixteen selected compounds interacted with urate oxidase (Uox) enzyme (PDB: 1R4U). When compared with the standard (ascorbic acid) it was found that 6-Propyl-5,6-dihydro-2H-pyran-2-one; Sydnone, 3-neopentyl-; Sedoheptulosan possessed the best docking interaction with a docking score of −5.509 kcal/mol, −5.141 kcal/mol, −5.015 kcal/mol respectively. The order of docking score for antioxidant activities was as followed: 6-Propyl-5,6-dihydro-2H-pyran-2-one > Sydnone, 3-neopentyl- > Sedoheptulosan > *p*-Mentha-1,4-dien-7-ol > α-Terpineol > 2-Mercaptopropionic acid >10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol > Butane, 1,3-Dichlorobutane > 1-Propanamine, 3-(methylthio)- > Methyl 11,12-tetradecadienoate. Nevertheless, 1-Dimethyl (prop-2-enyl)silyloxy-10-undecene; Silane, dimethyl (3-methylbut-3-enyloxy)isobutoxy-; Clofexamide; Acetic acid, (dodecahydro-7-hydroxy-1,4 b, 8,8-tetramethyl-10-oxo-2 (1 h); Chloroacetic acid, dodec-9-ynyl ester; Carbonic acid, but-3-yn-1-yl octadecyl ester didn't displayed any bonding interactions. Figure S2 and S3 represented the two-dimensional coordination of ligand-protein interaction for antioxidant activity. Also, the amino acid residues, which were linked with ligand-receptor interaction, had given in Table S1 for all pharmacological activity analyzed in this study.

3.9.1.2. Molecular docking for clot lysis activity. Table 5 represents the results of interacted compounds with tissue plasminogen activator receptor (PDB ID: 1A5H) for thrombolytic activity. The strongest docking interaction, which was even better than the standard (streptokinase), is exhibited by Sydnone, 3-neopentyl-; Sedoheptulosan; 10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol with a docking score of −6.972 kcal/mol, −5.798 kcal/mol, −5.243 kcal/mol. The order of docking score for thrombolytic activities was noted as: Sydnone, 3-neopentyl- > Sedoheptulosan >10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol > α-Terpineol > Clofexamide > *p*-Mentha-1,4-dien-7-ol > Butane, 1,3-Dichlorobutane > 2-Mercaptopropionic acid > 6-Propyl-5,6-dihydro-2H-pyran-2-one > 1-Propanamine, 3-(methylthio)- > Carbonic acid, but-3-yn-1-yl octadecyl ester > Methyl 11,12-tetradecadienoate > Chloroacetic acid, dodec-9-ynyl ester. Besides, 1-Dimethyl (prop-2-enyl)silyloxy-10-undecene; Silane,

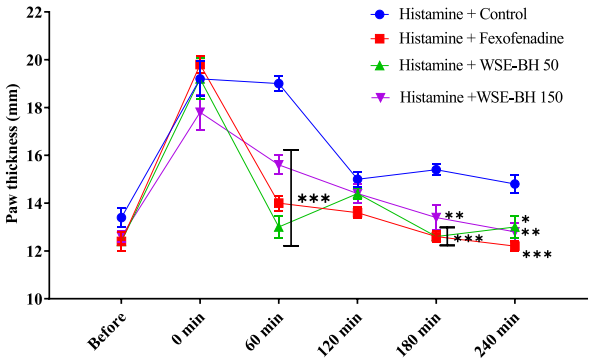

Fig. 11. Effect of WSE-BH and fexofenadine on histamine-induced paw edema in mice. The results were reported in the form of mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 are the levels of statistical significance via two-way ANOVA (Dunnett's test).

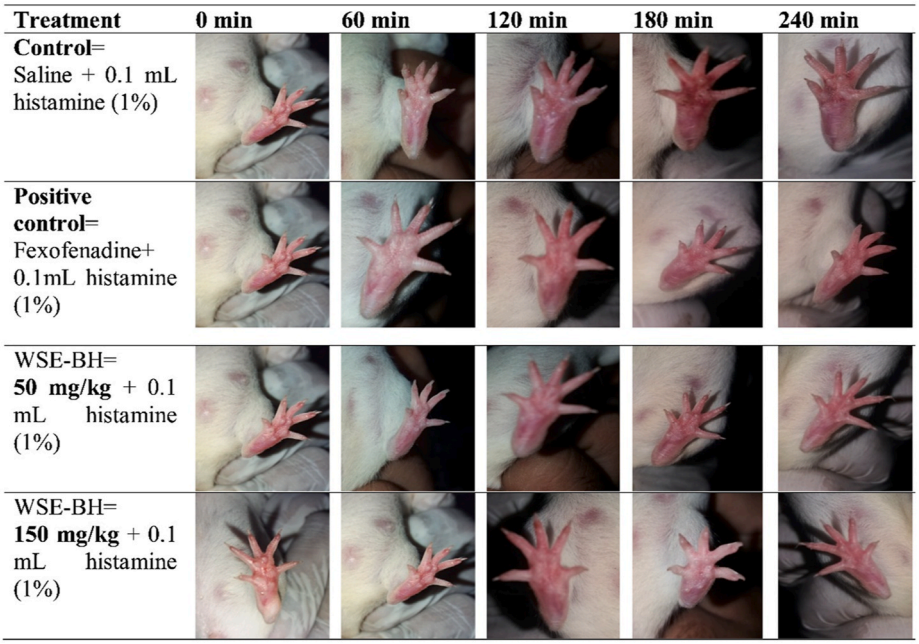

Fig. 12. Demonstration of histamine-induced paw edema in mice.

dimethyl (3-methylbut-3-enyloxy)isobutoxy-; Acetic acid, (dodecahydro-7-hydroxy-1,4 b, 8,8-tetramethyl-10-oxo-2 (1 h) didn't showed any bonding interactions. Figs. S4 and S5 showed the 2D representation of docking interaction for clot lysis activity.

3.9.1.3. Molecular docking for anxiolytic activity. Potassium channel (PDB: 4UUJ) were used in the molecular docking to determine anxiolytic activity of selected compounds. As shown in Table 5, the highest binding affinity observed for Sedoheptulosan; 2-Mercaptopropionic acid; and 6-Propyl-5,6-dihydro-2H-pyran-2-one with a docking score of −2.729 kcal/mol, −2.438 kcal/mol, and −2.186 kcal/mol, respectively. The docking scores analyzed were comparable to the standard agent (diazepam). The order of docking score for anxiolytic activities was noted as: 2-Mercaptopropionic acid > Sedoheptulosan > 6-Propyl-5,6-dihydro-2H-pyran-2-one > Carbonic acid, but-3-yn-1-yl octadecyl ester > Methyl 11,12-tetradecadienoate > Chloroacetic acid, dodec-9-ynyl ester. However, for 1-Propanamine, 3-(methylthio)-; Butane, 1,3-Dichlorobutane; Sydnone, 3-neopentyl-; 1-Dimethyl (prop-2-enyl)silyloxy-10-undecene; α-Terpineol; Silane, dimethyl (3-methylbut-3-enyloxy)isobutoxy-;10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol; Clofexamide; Acetic acid, (dodecahydro-7-hydroxy-1,4 b, 8,8-tetramethyl-10-oxo-2 (1 h); *p*-Mentha-1,4-dien-7-ol no bonding interactions were observed. Figure S6 showed the 2D representation of docking interaction for anxiolytic activity.

3.9.1.4. Molecular docking for antidepressant activity. Table 5 summarizes the results of the antidepressant molecular docking study. From 16 compounds *p*-Mentha-1,4-dien-7-ol; Sedoheptulosan; α-Terpineol unveiled the highest and binding affinity against human serotonin receptor (PDB: 5I6X) with a docking score of −6.802 kcal/mol, −6.232 kcal/mol and −6.129 kcal/mol respectively showing

Table 5
Molecular docking score of identified compounds from WSE-BH.

Compounds	Target Proteins and Docking scores (Kcal/mol)					
	1R4U	1A5H	4UUJ	5I6X	2OYE	6COX
1-Propanamine, 3-(methylthio)-	−3.416	−3.535	–	−4.661	−4.703	−4.097
Butane, 1,3-Dichlorobutane	−3.915	−4.538	–	−5.094	−5.391	−5.477
2-Mercaptopropionic acid	−4.381	−4.499	−2.438	−4.265	−4.314	−4.202
Sydnone, 3-neopentyl-	−5.141	−6.972	–	−5.771	−6.447	−6.442
α-Terpineol	−4.805	−4.914	–	−6.129	−5.988	−6.338
1-Dimethyl (prop-2-enyl)silyloxy-10-undecene	–	–	–	–	–	–
Silane, dimethyl (3-methylbut-3-enyloxy) isobutoxy-	–	–	–	–	–	–
10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol	−4.344	−5.243	–	−5.404	−6.762	−6.464
Clofexamide	–	−4.846	–	−5.154	−4.968	−5.139
Acetic acid, (dodecahydro-7-hydroxy-1,4 b,8,8-tetramethyl-10-oxo-2 (1 h)	–	–	–	–	–	–
Chloroacetic acid, dodec-9-ynyl ester	+1.289	−0.393	+2.404	−0.592	−2.103	−2.082
p-Mentha-1,4-dien-7-ol	−4.834	−4.762	–	−6.802	−6.441	−6.308
Methyl 11,12-tetradecadienoate	+1.751	−0.566	+1.914	−1.365	−1.769	+0.354
6-Propyl-5,6-dihydro-2H-pyran-2-one	−5.509	−4.43	−2.186	−4.983	−7.165	−7.073
Carbonic acid, but-3-yn-1-yl octadecyl ester	–	−2.121	−0.637	−3.421	−5.853	−4.741
Sedoheptulosan	−5.015	−5.798	−2.729	−6.232	−5.845	−6.357
Standard	**−4.655 (Ascorbic acid)**	**−4.53 (Strptokinase)**	**−3.035 (Diazepam)**	**−5.276 (Fluoxetine)**	**−5.977 (Aspirin)**	**−6.05 (Aspirin)**

better affinity towards the active site than the standard (fluoxetine). The order of docking score for antidepressant activities were *p*-Mentha-1,4-dien-7-ol > Sedoheptulosan > α-Terpineol > Sydnone, 3-neopentyl- > 10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol > Clofexamide > Butane, 1,3-Dichlorobutane > 6-Propyl-5,6-dihydro-2H-pyran-2-one > 1-Propanamine, 3-(methylthio)- > 2-Mercaptopropionic acid > Carbonic acid, but-3-yn-1-yl octadecyl ester > Methyl 11,12-tetradecadienoate > Chloroacetic acid, dodec-9-ynyl ester. Though, 1-Dimethyl (prop-2-enyl)silyloxy-10-undecene; Silane, dimethyl (3-methylbut-3-enyloxy)isobutoxy-; Acetic acid, (dodecahydro-7-hydroxy-1,4 b, 8,8-tetramethyl-10-oxo-2 (1 h) didn't showed any bonding interactions. Figure S7 and S8 showed the 2D representation of docking interaction for antidepressant activity.

3.9.1.5. Molecular docking for anti-inflammatory activity. To identify a plausible mechanism behind *Apis cerana indica* beehive inflammatory action, selected compounds were interacted with cyclooxygenase (COX)-1 (PDB ID: 2OYE), cyclooxygenase (COX)-2 (PDB ID: 6COX). For cyclooxygenase-1 and cyclooxygenase-2, the best the best docking score revealed by 6-Propyl-5,6-dihydro-2H-pyran-2-one (−7.165 and −7.073 kcal/mol, respectively) whereas Acetic acid, (dodecahydro-7-hydroxy-1,4 b, 8,8-tetramethyl-10-oxo-2 (1 h); Silane, dimethyl (3-methylbut-3-enyloxy)isobutoxy-; 1-Dimethyl (prop-2-enyl)silyloxy-10-undecene didn't showed any interaction at all. The order of docking score for cyclooxygenase-1 were 6-Propyl-5,6-dihydro-2H-pyran-2-one >10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol > Sydnone, 3-neopentyl- > *p*-Mentha-1,4-dien-7-ol > α-Terpineol > Carbonic acid, but-3-yn-1-yl octadecyl ester > Sedoheptulosan > Butane, 1,3-Dichlorobutane > Clofexamide > 1-Propanamine, 3-(methylthio)- > 2-Mercaptopropionic acid > Chloroacetic acid, dodec-9-ynyl ester > Methyl 11,12-tetradecadienoate. Moreover, for cyclooxygenase-2 the docking score were 6-Propyl-5,6-dihydro-2H-pyran-2-one >10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol > Sydnone, 3-neopentyl- > α-Terpineol > *p*-Mentha-1,4-dien-7-ol > Sedoheptulosan > Butane, 1,3-Dichlorobutane > Clofexamide > Carbonic acid, but-3-yn-1-yl octadecyl ester > 2-Mercaptopropionic acid > 1-Propanamine, 3-(methylthio)- > Chloroacetic acid, dodec-9-ynyl ester > Methyl 11,12-tetradecadienoate. These scores were compared and analyzed with a standard (aspirin). Consequently, the compounds with highest docking value were superior to the standard. All the results are demonstrated in Table 5. Figure S9 and S10 showed the 2D representation of docking interaction for cyclooxygenase-1 and Figure S11 and S12 depicted the interaction for cyclooxygenase-2.

3.9.2. ADME/T screening for drug-likeness

The pharmacokinetic features of the assigned compounds were measured using QikProp (Schrödinger v11.1), based on Lipinski's Rule of Five. Lipinski has indicated that if a drug/compound meets the following requirements, that the compound is likely to be orally bioavailable: molecular weight <500 amu; hydrogen bond donor sites <5; hydrogen bond acceptor sites <10; and lipophilicity value (LogP) < 5. Among the 19 compounds identified from *Apis cerana indica* beehive, four compounds, including 1-dimethyl (prop-2-enyl) silyloxy-10-undecene; chloroacetic acid, dodec-9-ynyl ester; methyl 11,12-tetradecadienoate; and carbonic acid, but-3-yn-1-yl octadecyl ester, violated Lipinski's Rule of Five. The toxicological properties revealed that butane, 1,3-dichlorobutane possesses carcinogenicity and Ames toxicity, whereas 1-propanamine, 3-(methylthio)-; 2-mercaptopropionic acid; acetic acid, (dodecahydro-7-hydroxy-1,4 b, 8,8-tetramethyl-10-oxo-2 (1 h); and chloroacetic acid, dodec-9-ynyl ester are carcinogenic but non-Ames toxic. Based on the findings in Table 6, 11 compounds can be termed as potential lead compounds with high bioavailability and could be promising

candidates for therapeutic development.

4. Discussion

Various civilizations and cultures have long employed plant-based natural products and herbal extracts to treat multiple disorders. These plant-derived remedies and dietary supplements used to treat mild to moderate diseases have recently become the subject of modern investigations to ascertain the definite pharmacological effects associated with the products to identify those that might be beneficial for human use [44]. The current study corroborated both *in vitro* and *in vivo* evidence of the biological activities of *Apis cerana indica* beehive extracts by performing computational analysis to examine how the bioactive components exert effects against neuropsychiatric and inflammatory disorders by suppressing oxidative stress signaling. The present study identified 19 phytocompounds in WSE-BH with significant thrombolytic properties. Additionally, three different extracts were analyzed to identify potentially bioactive compounds in beehive. WSE-BH, when administered to mice at doses of 50 mg/kg and 150 mg/kg, demonstrated extrinsic antidepressant and anxiolytic activity, in addition to significant anti-inflammatory activity, possibly mediated by reducing histamine release.

Diverse new drug components have been identified from metabolites sourced from natural plants using a variety of powerful analytical techniques. GC-MS is a well-established technology with high sensitivity, high resolution, good reproducibility, relatively low cost, with a large standard library [45]. GC-MS/MS was used in the current investigation to separate and identify bioactive compounds and secondary metabolites found in *Apis cerana indica* beehive. Based on the retention times, several compounds, such as 2H-Pyran-2-one, 5,6-dihydro-6-pentyl [46]; sydnone, 3-neopentyl- [47]; sedoheptulose, α-terpineol [48]; ephedrine; and clofexamide, were identified in the current experiment.

The metabolic activities of the living body play prolific roles in the generation of ROS and non-radical molecules, including OH^{-}, H_2O_2, and O_2, which might help to strengthen cellular tolerance to diverse environmental stresses and the induction of programmed cell death. However, excessive ROS production is harmful to the body and can lead to oxidative stress [49]. Oxidative stress describes a condition in which oxidant level overwhelms the antioxidant protective system, leading to cellular lipid peroxidation and DNA damage [50]. In general, due to the ability to scavenge free radicals, phenolic and flavonoid compounds can improve cellular stability by suppressing oxidation or improving antioxidant activity. Phenolic and flavonoid compounds feature aromatic structures, and modification, such as the placement of hydroxyl groups (–OH) in the aromatic ring, can interfere with electron transfer in the compound due to hydrophilicity, whereas methoxy groups can improve or suppress anti-oxidative activity by modifying structural planarity and increasing hydrophobicity. Carbonyl groups (C═O) in aromatics can also expand the resonance effects of a compound [51]. Correspondingly, phytochemicals can scavenge free radicals by forming proton ions [H^{+}], and the photochemical compounds can recover their structural balance through electron transfer from their own oxygen-based functional groups or the aromatic structures modified by free radicals [19,51–53]. The TPC, TFC, and DPPH free radical scavenging activity of WSE-BH were tested in this ongoing investigation. WSE-BH was found to be rich in both TPC and TFC, with a moderate antioxidant effect (IC_{50} = 293.52 μg/mL). In the molecular docking test, 2H-pyran-2-one, 5,6-dihydro-6-pentyl, which is a known flavoring agent, showed the best docking interaction. Therefore, the presence of flavoring agents with aromatic structures in WSE-BH may be responsible for the free radical scavenging

Table 6
ADME/T and toxicological properties prediction of the bioactive compounds.

Compounds	ADME/T				Toxicological properties			
	MW (<500 g/mol)	HBA (<10)	HBD (<5)	Log P (≤5);	nRB (≤10)	Ames Toxicity	Carcinogens	AOT
1-Propanamine, 3-(methylthio)-	105.2	2	1	0.3	3	NAT	CA	II
Butane, 1,3-Dichlorobutane	127.01	0	0	2.2	2	AT	CA	III
2-Mercaptopropionic acid	106.15	3	2	0.5	1	NAT	CA	II
Sydnone, 3-neopentyl-	157.19	3	1	1.9	2	NAT	NCA	III
α-Terpineol	154.25	1	1	1.8	1	NAT	NCA	IV
1-Dimethyl (prop-2-enyl)silyloxy-10-undecene	268.51	1	0	4.50	13	NAT	NCA	III
Silane, dimethyl (3-methylbut-3-enyloxy)isobutoxy-	216.39	2	0	3.72	7	NAT	NCA	III
10-(Tetrahydro-pyran-2-yloxy)-tricyclo [4.2.1.1 (2,5)]decan-9-ol	252.35	3	1	2.2	2	NAT	NCA	III
Clofexamide	284.78	3	1	2.6	8	NAT	NCA	III
Acetic acid, (dodecahydro-7-hydroxy-1,4 b,8,8-tetramethyl-10-oxo-2 (1 h)	405.6	5	1	3.1	5	NAT	CA	III
Chloroacetic acid, dodec-9-ynyl ester	258.78	2	0	5.1	10	NAT	CA	II
p-Mentha-1,4-dien-7-ol	152.23	1	1	1.5	2	NAT	NCA	III
Methyl 11,12-tetradecadienoate	238.37	2	0	4.6	11	NAT	NCA	III
6-Propyl-5,6-dihydro-2H-pyran-2-one	140.18	2	0	2	2	NAT	NCA	III
Carbonic acid, but-3-yn-1-yl octadecyl ester	366.6	3	0	9.8	21	NAT	NCA	III
Sedoheptulosan	192.17	6	4	−2.5	1	NAT	NCA	IV

Here, MW, Molecular weight (g/mol); HBA, Hydrogen bond acceptor; HBD, Hydrogen bond donor; Log P, Lipophilicity; nRB: number of rotatable bonds; TPSA: topological polar surface area. AOT: Acute Oral Toxicity.
NM: Non-mutagenic; NC: Non-carcinogenic; NH: Non-hepatotoxic; Class I (LD50 ≤ 5); Class II (5 < LD50 ≤ 50); Class III (50 < LD50 ≤ 300); Class IV (300 < LD50 ≤ 2000); Class V (2000 < LD50 ≤ 5000); Class VI (LD50 > 5000).

abilities of WSE-BH by interfering with electron transfer and donating proton ions [H^+] [46].

The formation of blood clots in blood vessels represents a prominent sign of major diseases, such as acute ischemic stroke. Oxidative stress is known to contribute to the development of arterial blockage, leading to acute ischemic stroke. The disproportionate generation of ROS causes malondialdehyde to form in the endoplasmic reticulum, activating matrix metalloproteinases, which alter the functions and structures of proteins [54]. An imbalance in matrix metalloproteinase activity can trigger protein synthesis and extracellular matrix degradation, and the activation of matrix metalloproteinase-9 has been connected with the development of blood clots and arterial blockage in a rodent model of ischemic stroke [55]. Streptokinase is a readily available therapeutic agent for the treatment of ischemic stroke, as demonstrated by the Multicenter Acute Stroke Trial and Australian Streptokinase [ASK] trial, which reestablishes blood flow in vessels by dissolving the clot. Streptokinase was used in the current investigation as a positive control for clot lysis [52,56]. WSE-BH extract demonstrated significant clot lysis activity, reducing clot size by 60.06% compared with the control group, whereas streptokinase achieved a 72.97% reduction. WSE-BH might exert this potent antithrombotic activity through the reduction of endoplasmic reticulum stress via the inhibition of protein synthesis and the elimination of macrophages in atherosclerotic plaques, due to the presence of sydnone, 3-neopentyl-, which showed the strongest docking scores during the molecular docking phase of the current experiment [47].

The hierarchical oxidative stress theory proposes a system in which decreased levels of oxidative stress correlated with cytoprotective activities, such as those involving detoxification enzymes and antioxidants, whereas high levels of oxidative stress cause cell damage or even death [57]. Increased oxidative stress has been reported to induce the formation of advanced glycation end-products (AGEs), inducing cytotoxicity. There was a significant correlation among the brine shrimp lethality bioassay of cytotoxicity and the *in vitro* growth inhibition of human solid tumor cell lines, which suggests that the brine shrimp lethality bioassay can be used as an important screening test for antitumor drug research (National Cancer Institute, USA) [58]. In this assay, an increased LC_{50} is associated with reduced extract toxicity [52]. The water-soluble extract of *Apis cerana indica* beehive showed a non-toxic effect (2143.99 μg/mL) in the brine shrimp lethality bioassay. A previous *in vivo* study used WSE-BH in an acute toxicity study, which showed that this extract is safe up to a dose of 4000 mg/kg [22]. Further in-depth investigations of *Apis cerana indica* beehive extract use in human clinical trials remain necessary to verify the safety of this natural product.

Anxiety is the most common psychiatric disorder, with a significant morbidity rate [59]. Several lines of evidence have proposed that oxidative stress may play a role in the management of anxiety by altering neuronal plasticity. In general, oxidative stress significantly increases the levels of mitochondrial $O^{2\bullet-}$ in the brain and the accumulation of H_2O_2, which react with mitochondrial Fe^{2+} to form the highly oxidizing hydroxyl radical (HO), leading to changes in neurotransmitters and causing anxiety [60]. Phytochemicals are known to detoxify H_2O_2 by replacing hydrogen with methyl, acetyl, and hydroxyl groups [61]. Although the underlying biological responses of these phytochemicals are determined by their bioactive constituents, analyzing the crude extracts is also important. The anxiolytic properties of WSE-BH were assessed using two well-known models: HBT and EPM. The anxiolytic response in the HBT is measured by assessing the head-dipping behavior, whereas the number of entries and time spent in the open arms of the EPM are used to assess anxiety. The administration of WSE-BH at a dose of 150 mg/kg resulted in a significant increase ($p < 0.01$) in the number head-dipping events in the HBT and an increase in both the number of entries and time spent in the open arms in the EPM, indicating significant anxiolytic activity. These findings are similar with previous reports, indicating that WSE-BH as a potent anxiolytic candidate [22]. In the computer-assisted drug design phase of this research, sedoheptulosan, which an anhydride of sedoheptulose, exhibited the best docking score (−2.729 kcal/mol). Further in-depth investigations to determine the exact mechanistic roles played by WSE-BH in the treatment of anxiety are worthwhile.

Oxidative stress is known to result in the increased release of ROS, which have been reported to cause striking reductions in the number and density of glial cells in post-mortem samples and preclinical animal models [62]. These alterations can modulate the hypothalamic–pituitary–adrenal (HPA) axis feedback and alter serotonergic and γ-aminobutyric acid (GABA) transmission, promoting the release of corticotropin-releasing factor (CRF) from the neurons in the periventricular nucleus (PVN) [63]. The dysregulation of CRF increases the level of plasma cortisol and cerebrospinal fluid and suppresses adrenocorticotropic hormone (ACTH) release [64]. Clinically effective antidepressants have been reported to stimulate antioxidant enzyme activities, restoring the function and expression of CRF and the regulation of the HPA axis, which is manifested as anti-immobility effects in preclinical models of depression, such as the TST or FST [65,66]. The antidepressant effects of WSE-BH were evaluated, and the results were similar to those for the selective serotonin reuptake inhibitor fluoxetine. In both the TST and FST models, the WSE-BH doses of 50 and 150 mg/kg demonstrated significant ($p < 0.001$) reductions in the immobility time, as did fluoxetine, compared with the control group. The antidepressant effects displayed by WSE-BH might be due to the presence of α-terpineol, which exhibited the best docking score (−6.129 kcal/mol) and has been reported to restore the HPA axis function by modulating monoamine neurotransmitter levels [48].

Inflammation by carrageenan-induced paw edema is highly reliable, as this model causes both acute and local inflammatory responses in the paw tissues. Tissue injury leads to the activation of COX and lysyl oxidase (LOX), resulting in the formation of cytotoxins, prostanoids, and leukotrienes, which act in hypernociceptive signals [67]. However, carrageenan-induced edema is known to manifest as a biphasic event, with the initial phase starting within 1 h of carrageenan induction, whereas the second phase occurs after 2–3 h [68]. The initial phase is thought to be associated with the release of histamine, bradykinin, serotonin, thrombin, and vascular endothelial growth factor, whereas the second phase is thought to be due to the local infiltration of neutrophils, prostaglandins, oxygen-derived free radicals, and NO [7,69]. Surprisingly, both doses of WSE-BH showed significant ($p < 0.001$) effects in reducing edema compared with the control group at 60 min and remained significantly effective after 240 min ($p < 0.5$, $p < 0.01$). These results clearly indicate that WSE-BH might reduce histamine, bradykinin, serotonin, thrombin, or vascular endothelial growth factor release during the acute phase of edema and may also decrease the local infiltration of neutrophils, prostaglandins, oxygen-derived free radicals, or nitric oxide during the chronic phase of edema. The GC-MS analysis identified α-terpineol, which showed a strong binding

affinity for COX-1 and -2 receptors (−5.988 and −6.338 kcal/mol, respectively) in the molecular docking experiment [70,71].

Bioinformatics tools are increasingly used by pharmaceutical companies to perform structure-based drug discovery, as these tools can be used to determine new ligands for proteins with identified structures, based on preliminary reports obtained from *in vivo* and *in vitro* analysis within less cost and shorter times than typical drug discovery methods [52]. Computer docking tools are used by researchers worldwide to identify and analyze the binding affinities of molecules that fit the binding sites of proteins. Molecular docking experiments using the components identified in different medicinal plant species are increasingly used in structure-based drug design to anticipate the intermolecular complexes formed between medicinal molecules and their target proteins [72]. The characterization of pharmacokinetic (ADME/T) and toxicological properties must also be performed prior to the commercialization of medicines, which can also reduce the costs of drug development. In the present experiment, 19 bioactive compounds were identified by the GC-MS/MS analysis of *Apis cerana indica* beehive extracts, and 16 compounds were selected to examine bioactivity against various target proteins in the molecular docking assay. Sedoheptulosan might represent a lead compound because it exhibited strong interactions with all of the tested target proteins, satisfied Lipinski's Rule of Five, and displayed non-Ames toxicity and non-carcinogenicity. This study provides better knowledge of the potential uses of WSE-BH by comparing the pharmacokinetic and pharmacodynamic characteristics with experimental results, which can guide the next phases of investigation prior to clinical trial.

5. Conclusions

This study found that components derived from *Apis cerana indica* beehive display significant anxiolytic, antidepressant, and anti-inflammatory properties with antioxidant potential. Additionally, WSE-BH demonstrated significant thrombolytic and cytotoxic activities. In the molecular docking studies, several bioactive compounds identified in WSE-BH revealed binding affinities against different proteins involved in these bioactive pathways. The identified active compounds were found to have drug-like properties and were characterized as safe in ADME/T and toxicity investigations. Therefore, beehive-derived products might be investigated as dietary supplements for the management of neurodevelopmental disorders. To determine whether beehive-derived products display neuroprotective properties, more mechanistic studies, and dose–response studies are highly recommended.

Author contribution statement

Abu Montakim Tareq: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Md Mohotasin Hossain: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Analyzed and interpreted the data.

Main Uddin, Farhanul Islam, Zidan Khan, Chadni Lyzu: Performed the experiments.

Md Mobarak Karim, Talha Bin Emran, A.S.M. Ali Reza: Analyzed and interpreted the data: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Duygu Ağagündüz: Contributed reagents, materials, analysis tools or data.

Raffaele Capasso: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

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.

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Appendix A. Supplementary data

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