



CNS anti-depressant, anxiolytic and analgesic effects of *Ganoderma applanatum* (mushroom) along with ligand-receptor binding screening provide new insights: Multi-disciplinary approaches

S M Moazzem Hossen^{a,*,**}, Mohammad Jahedul Islam^a, Md. Rabiul Hossain^b, Anik Barua^c, Md. Giash Uddin^a, Nazim Uddin Emon^{d,*}

^a Department of Pharmacy, Faculty of Biological Science, University of Chittagong, Chittagong, 4331, Bangladesh

^b Department of Pharmacy, University of Science and Technology Chittagong, Chittagong, 4202, Bangladesh

^c Department of Biochemistry and Biotechnology, University of Science and Technology Chittagong, Chittagong, 4202, Bangladesh

^d Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Chittagong, 4318, Bangladesh

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ABSTRACT

This research was designed to evaluate the CNS depressant, anxiolytic, and analgesic action of aqueous and ethanol extract of *Ganoderma applanatum*, a valuable medicinal fungus used in multiple disorders belongs to Ganodermataceae family. Two extracts of *G. applanatum* were prepared using distilled water and ethanol as solvents and named AEGA and EEGA. Open field method, rotarod method, tail suspension method, and hole cross method were utilized for the CNS depressant action. In contrast, elevated plus-maze test and hole board method were utilized for the anxiolytic action. For determining the analgesic potential, acetic acid-induced writhing test, hot plate method, and tail immersion test were used. Besides, molecular docking has been implemented by using Discovery studio 2020, UCSF Chimera and PyRx autodock vina. At both doses (200 and 400 mg/kg) of AEGA and EEGA showed significant CNS depressant effect ($p < 0.05$ to 0.001) against all four tests used for CNS depressant activity. Both doses of AEGA and EEGA exhibited important anxiolytic activity effect ($p < 0.05$ to 0.001) against the EPM and hole board test. Both doses of AEGA and EEGA also exhibited a potential analgesic effect ($p < 0.05$ to 0.001) against all three tests used for analgesic action. In addition, in the molecular docking the compounds obtained the scores of -5.2 to -12.8 kcal/mol. Ganoapplanin, sphaeropsidin D and cytosporone C showed the best binding affinity to the selected receptors. It can be concluded that AEGA and EEGA have potential CNS depressant, anxiolytic, and analgesic action, which can be used as a natural antidepressant, anxiolytic, and analgesic source.

1. Introduction

Depression and anxiety are frequently found in mental disorders due to lifestyle stress [1]. Day by day, the number of patients suffering from mental disorders have been expanding, remarkably in emerging nations [2]. According to the report of the WHO, approximately 322 million persons are experiencing melancholy. This report also includes that anxiety disorders affect nearly 3.6% of the global people (more than 260 million people) [3]. Anxiety and depression can be influenced by genetic, epigenetic and environmental factors which orient further

pathophysiological complexes [4]. Among the psychiatric disorders, anxiety and depression are the most common illness and together they are highly considered to the part of the wider internalizing condition. Based on data from Mental Health Care Administration and Alcohol Abuse, in 2017 the 12-month incidence of major depression disease was measured at 7.1% for adults and 13.3% in youth among the total population [5]. Currently, several antidepressant and anxiolytic medications are attainable from the market. They are still associated with various side effects, including sexual dysfunction, hypotension, gaining weight, sleeping difficulty, physical dependence, tolerance, and

Abbreviations: WHO, World health organization; NSAID, Nonsteroidal anti-inflammatory drugs; AEGA, Aqueous extract of *G. applanatum*; EEGA, Ethanol extract of *G. applanatum*; MPE, Maximum possible effect; PRT, Pain reaction time.

* Corresponding author. Department of Pharmacy, Faculty of Science and Engineering, International Islamic University Chittagong, Chittagong, 4318, Bangladesh.

** Corresponding author. Department of Pharmacy, University of Chittagong, Chittagong, 4331, Bangladesh.

E-mail addresses: hossen.pharmacy@cu.ac.bd (S.M.M. Hossen), nazim7emon@gmail.com (N.U. Emon).

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cardio-toxicity [6].

The severity, consistency, and period of pain can differ greatly and have different pathophysiological mechanisms and definitions. The clear and succinct description of the idea of pain is, therefore a challenge [7]. Pain is a psychic, sensory, and obnoxious event that mostly results from tissue injury [8]. Although analgesics like NSAIDs and opiates are frequently utilized to alleviate the pain without modifying sentience, they are liable for generating various side effects [9]. Ulceration, bleeding, gastrointestinal irritation, etc. are produced by NSAIDs, while opiates frequently create dependence and tolerance [9]. For this reason, researchers have shown concern to discover analgesic drugs from natural origins with fewer side effects [10].

Computational biology represents the immense opportunity that biologists are currently generating and validate experiments through this approach. The computer-aided drug development (CADD) techniques and molecular docking have been demonstrated under *in silico* process to develop and investigate drug design within a limited period [11]. A successful molecular docking should be able to establish the status of ligands on the binding site and the physicochemical relationship of the protein structure [12].

Drugs from natural origins have few side effects, but they are comparable in their ability to treat diseases as the synthetic derivate. Searching for alternative drug therapies for psychiatric illness from mushroom sources are recently improved dramatically [13,14]. Medicinal plants are a promising source of life-saving therapeutic products worldwide, owing to their diverse pharmacological functions [15].

Ganoderma is also identified as reishi (in Japan) or lingzhi (in China), relating to the family Ganodermataceae, scattered throughout the world [16]. Over 250 species of this fungal genus are identified. Unique double-walled basidiospores are the characteristic of this fungal genus [17,18]. Latterly pharmaceutical industries have shown concern in Ganoderma species because of their enormous folkloric medicinal uses for thousands of years [19]. *Ganoderma applanatum* is a wood-decay fungus and generally found at the bases of tree stumps [20]. The different parts of the *G. applanatum* contains Applanoxidic acid E, Applanatumol A, Applanatumin A, Applanatumol E, Applanatumol P, Applanatumol Q, Cytosporone C, Stemphone B, Condidymic acid, Nigragillin, Ganoapplanin, Myrocin C, Sphaeropsidin D, Epoxy-4,4, 14-trimethyl-3,7,11,15,20-penta-oxo-pregnane, Comazaphilone D, Applanoxidic acid (C-G), and etc; as its biochemical compositions [20–23]. Several studies represented that *G. applanatum* contains compounds with potent anti-tumor, antibacterial, and antifibrotic properties [24]. Various medicines have been prepared from this fungus to treat the liver injury, cirrhosis, and hepatitis [25]. *G. applanatum* is also found to have antiobesity, antiviral, antioxidative, and immunomodulating activities [20]. Therefore, in this study, *in vivo* antidepressant, anxiolytic, and analgesic effects of the two extract of *G. applanatum* along with phytochemical evaluation were analyzed. Besides, computational investigation (molecular docking) of the chemical composition with the receptors associated with the antidepressant, anxiolytic, and analgesic effects also have been implemented as these studies were not analyzed previously.

2. Materials and methods

2.1. Drugs and chemicals

Analytical grade chemicals and solvents were utilized during this experiment. Diclofenac sodium and diazepam were purchased from the Bangladeshi pharmaceutical company named Square Pharmaceuticals Limited. Department of Pharmacy, University of Chittagong, had provided the remaining chemicals.

2.2. Experimental animal

4–5 weeks old albino rodents of both genders within 20–25 g weight

range were obtained from BCSIR, Chattogram, Bangladesh. They remained housed in room temperature conditions for a week before commencing the test to acclimatize the environment. During the experimental period, a balanced diet and tap water were provided to the Swiss albino mice.

2.3. Animal euthanasia

The animals were treated in compliance with the values of the Swiss Academy of Sciences and Swiss Academy of Medical Sciences and have been euthanized according to the recommendations for animal euthanasia: 2013 edition [26]. This research has been approved by the ethical review board of the Department of Pharmacy, University of Chittagong, Bangladesh under the consent number: CUDP:March 16, 2019:17).

2.4. Collection and identification of the plant

The mushroom was identified by fungus expert Dr. Akther Jahan Kakon, Mushroom Specialist, Mushroom Development Institute, Savar, Dhaka, and systemic herbarium was prepared for authentication and further correspondence if necessary. The identified mushroom is *G. applanatum*. Herbarium Accession number: 2018/004/Fungi/CU/DP.

2.5. Preparation of the plant extract

The gathered mushrooms were milled into the powders for effective extraction. The powders were entered into a clean glass container for soaking in solvents. The sealed vessel was retained for 14 days with occasional shaking. Whatman filter paper no. 1 was used to filter the mixture while a rotary evaporator was used for the evaporation of the solvents. Finally, the blackish crude extracts were obtained and kept in the refrigerator for further use. Two extracts of *G. applanatum* were prepared by using distilled water and absolute ethanol as solvent named as the aqueous extract of *G. applanatum* (AEGA) and ethanol extract of *G. applanatum* (EEGA).

2.6. Phytochemical screening

Standard methods were adopted for the qualitative determination of active constituents present in the crude ethanol and aqueous extract of *G. applanatum* [27,28].

2.7. CNS anti-depressant activity of the plant extracts

2.7.1. Open field test

For assessing the CNS depressant activity, the process illustrated by Gupta and Hussain et al. [29,30] was used with a small adjustment. After splitting the animals into six classes (n = 5), negative control (1% Tween-80 in saline, 10 mL/kg; p. o.), positive control (diazepam, 1 mg/kg, i. p.) AEGA and EEGA (200 and 400 mg/kg; p. o.) was administered to different classes. The number of fields covered by individual mice of different groups was noted for 3 min at 0, 30, 60, 90, 120 min throughout the experimental phase.

2.7.2. Rotarod test

Dunham and Miya outlined the method used for this experiment with small adjustments [31]. A rotarod apparatus with a four-section design was used to perform this research. Rodents were split into six classes (n = 5), and each class was managed with similar doses, as mentioned in the previous method. The extracts were orally administered 60 min before commencing the test, while diazepam was intraperitoneally administered 30 min before starting the experiment. The time at which the animals fell off from the rotating rod at a speed of 24 rpm was noted at 30, 60, and 90 min.

2.7.3. Tail suspension test

This test was performed with slight modifications. In this experiment, rodents were divided into six classes, with five mice in any class. The extracts of *G. applanatum* (200 and 400 mg/kg, p. o.) was used to treat the test groups (Group III, Group IV, Group V, and Group VI). The diazepam (1 mg/kg, i. p.) was used as a positive control, while 1% Tween-80 in saline was negative (10 mL/kg, p. o.). Thirty minutes after the drug administration to the animals, rodents were hung from 50 cm above the ground, utilizing sticky tape lodged nearly 1 cm from their tails' apex. The period during which the immobility of animals recorded was 6 min. When the mice silently hung or remained motionless, they were considered immobile [32].

2.7.4. Hole cross test

The process was performed as defined by Islam et al. [33]. Group I received 1% Tween-80 in saline orally, while Group II received diazepam (1 mg/kg, i. p.). On the other hand, the remaining experimental groups received *G. applanatum* extracts at the doses of 200 mg/kg and 400 mg/kg body weight orally. The number of holes crossed by the mice in 3 min was recorded at 0, 30, 60, 90, and 120 min [34].

2.8. Anxiolytic activity of the plant extracts

2.8.1. Hole board test

The hole board device formed of a wooden chamber (40 × 40 × 25 cm) with 16 equally aligned holes (3 cm diameter). The device was constructed to a climax of 25 cm from the ground so that the mice could peep through the holes. The rodents were treated with *G. applanatum* (200 and 400 mg/kg, p. o.), the vehicle (10 mL/kg, p. o.) or diazepam (1 mg/kg, i. p.), 30 min before commencing the experiment. The numbers and the duration of the head poking of each animal were counted during the 5 min observation period [35].

2.8.2. Elevated plus-maze test

This widely approved experiment was conducted for evaluating anxiolytic activities in rodents [36]. The elevated plus-maze was positioned 50 cm above the ground. The animals were split into negative control, positive control, and test groups (n = 5). After treating the test extracts (200 and 400 mg/kg), diazepam (1 mg/kg), and vehicle, every mice was located in the middle of the maze approaching the closed arms. The time spent in closed and open arms and the total numbers of open and closed arm entries were noted for 5 min at 0, 30, 60, 90, and 120 min. The entire operation was managed in a sound-attenuated place.

2.9. Analgesic activity of the plant extract

2.9.1. Acetic acid-induced writhing test

The acetic acid-induced writhing method was widely used to evaluate the analgesic potential of *G. applanatum* [37,38]. After overnight fasting, two different doses (200 and 400 mg/kg body weight) of the test extracts, vehicle (10 mL/kg body weight), diclofenac sodium (10 mg/kg body weight) were treated orally 40 min before the intraperitoneal injection of 0.7% acetic acid. The total numbers of writhing were counted for each animal for 15 min, 5 min after acetic acid administration.

2.9.2. Hot plate test

When the rodents were put on Eddy's hot plate [39], the heat was kept at 55 ± 0.5 °C. To avoid the harmful effect on mice's skin, a cutoff time of 30 s was maintained [40]. After oral treatment of the specimens, the response period was noted at 0, 30, 60, and 90, 120, 180, and 240 min. The experiment groups' mice were treated at two doses (200 and 400 mg/kg body weight). The positive control group received diclofenac sodium (10 mg/kg body weight) while the negative control received

vehicle (10 mL/kg body weight).

2.9.3. Tail immersion test

The tail immersion method was done to assess the central analgesic property and followed the developed method mentioned by Gawel et al. [41]. This method is based on the outcome that drugs that show a morphine-like effect can extend the abandonment of the mice tail from warm water. After treating the animal with diclofenac sodium or extracts, one to two cm of the rodents' tail was submerged in hot water, maintaining a temperature of 54 ± 0.5 °C. After administering the drug and extracts, the tail-withdrawal reaction's latency duration was recorded at 30, 60, 90, and 120 min. The latency phase was kept to 20 s so that the damage of tail tissue could be bypassed.

2.10. In silico computer aid virtual screening

2.10.1. Software tools

During the process of this analysis, the following were used: UCSF Chimera, AutodockVina, Discovery Studio Visualizer 2020 (BIOVIA), drug banking, MGL instruments, Protein Data Bank (PDB) and PubChem.

2.10.2. Selection of the target proteins

A systematic review for the current scientific research has mainly accomplished the collection of possible treatment objectives of anxiety, depression and pain. From the protein data bank (PDB) (large biological molecules database viz.), the selected targets were confirmed again [42].

2.10.3. Preparation of target proteins

Cyclooxygenase-1 (PDB: 2OYE) [43], Potassium channel KCSA-FAB (PDB: 4UUJ) [44] and human serotonin transporter (PDB: 5I6X) [45] have been downloaded from the RCSB Protein Data Bank (PDB). These constructs have been identified as acceptable and connected to *Homo sapiens*, *Mus musculus*, *Streptomyces lividans* and *Ovis aries*. In addition, these structures were also used in recent research works and have been found in a various reputed published journal. This ensures that the findings are reproducible by using relevant tools. PDB ID 2OYE, 4UUJ and 5I6X are well-authenticated receptors for activated pain, anxiety and depression. This ensures that the findings are reproducible by using relevant tools. PDB ID 2OYE, 4UUJ and 5I6X are well-authenticated receptors for activated pain, anxiety and depression. Beginning of the study, the protein ligands were detached from the complexes via Discovery Studio Visualizer (BIOVIA). For optimization and preparation of these proteins in the docking process, UCSF Chimera has been used. Water molecules were eliminated from structures and polar hydrogen was added to the necessary H-atoms. After the gasteiger charge estimation, these proteins were converted and saved into pdbqt format with AutodockVina docking [46].

2.10.4. Virtual filtering

To complete the virtual screening, PyRx-autodockvina software has been operated [46]. The ligands (Applanatumol E, Cytosporone C, Nigragillin, Ganoapplanin, Myrocin C, Sphaeropsidin D) of *Ganoderma applanatum* mushroom (Fig. 1) were minimized and optimized by PyRx-autodock vina software and were docked with the selected receptor targets (2OYE, 4UUJ and 5I6X). The implementation of the PyRx autogrid program, ligand centered grid box has covered the binding site of the target pockets of the receptors. The selection of the grid box enables the ligand to pass about freely within the allocated values in planes (X, Y and Z). The coordinate values of x, y and z were obtained using the Discovery Studio Visualizer from the respective PDB crystals, i.e. 2OYE, 4UUJ and 5I6X. Grid scale was 60 Å × 60 Å × 60 Å as in previous

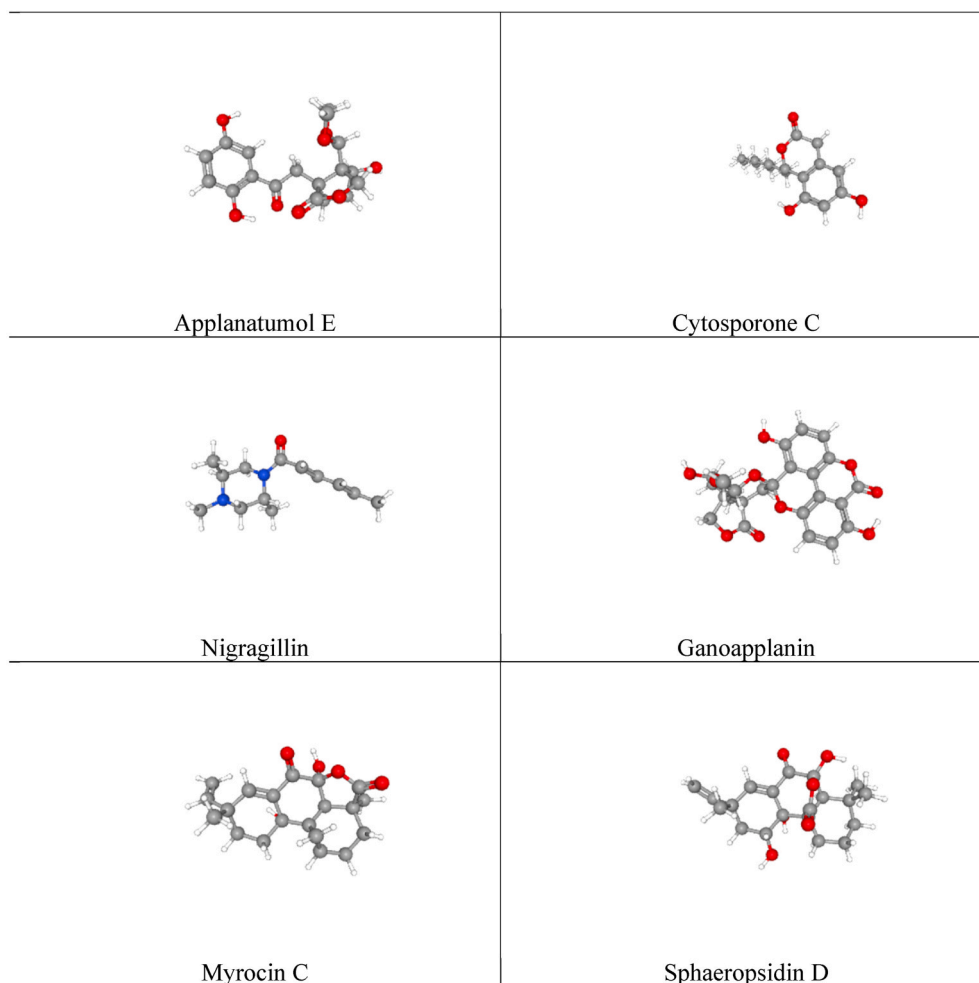


Fig. 1. The chemical structures of the selected compounds (Applanatumol E, Cytosporone C, Nigragillin, Ganoapplanin, Myrocin C, Sphaeropsidin D) of *Ganoderma applanatum* mushroom.

publications. Finally, the docking (ligand-protein interactions) have been visualized by BIOVIA Discovery Studio Visualizer 2020.

3. Statistical analysis

The data were manifested as mean \pm SEM. Statistical comparisons were conducted using one-way ANOVA followed by Dunnett's multiple comparison tests. The resulting values were compared with the positive control group and were statistically significant when $p < 0.05$, $p < 0.01$, $p < 0.001$.

4. Results

4.1. Phytochemical screening

The results of the phytochemical screening has been presented in [Table 1](#). The screening showed the presence of Alkaloids, Glycosides, Triterpenes, Carbohydrate, Flavonoids and Steroids in the ethanol extract of the *G. applanatum*. Besides, the primary screening of the aqueous extract of the *G. applanatum* yielded the presence of Alkaloids, Cardiac glycosides, Triterpenes, Carbohydrate, Flavonoids and Steroids.

Table 1

Observation and results of chemical screening.

Secondary Metabolites	Name of the test	Observation	Result	
			EEGA	AEGA
Alkaloids	Wagner's test	Brown or deep brown precipitate	+	+
Glycosides	General test	Yellow color	+	-
Cardiac glycosides	1. Legal's test	1. No pink or red color	-	+
	2. Baljet's test	2. No yellow orange color		
Triterpenes	Salkowsky test	Reddish coloration	+	-
Carbohydrate	Molisch test	Red-violet layer at the interface between the acid (bottom) and aqueous (upper) layers	+	+
Flavonoids	1. General test	1. A yellow coloration	+	+
	2. Specific test	2. Orange to red color		
Steroids	Libermann-Burchard's test	Greenish color	+	+
Tannins	FeCl ₃ test	No brownish green color	-	-
Saponins	Frothing test	Change is observed	-	-

“+” stands for the presence and “-” indicates the absence of secondary metabolites.

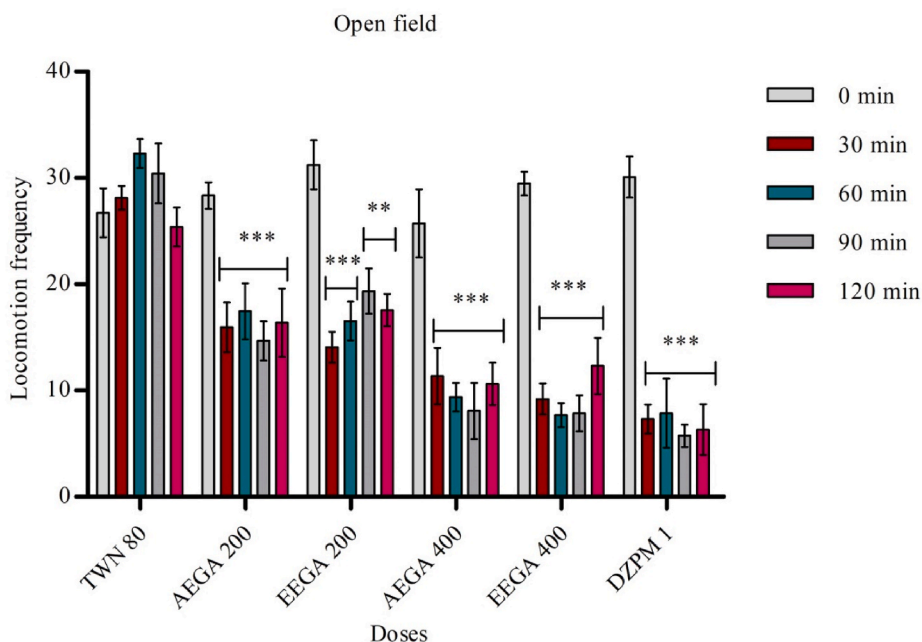


Fig. 2. Effect of test samples in open field test. Results are expressed as mean ± SEM (n = 5) and statistically analyzed using ANOVA followed by Dunnett’s comparison test. *p < 0.05, **p < 0.01 ***p < 0.001 have been considered as significant, when compared with control (n = 5). AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

4.2. Open field test

Both extract (water and ethanol) of *G. applanatum* fungus were subjected to screening for the open-field method’s anxiolytic activity. The test was performed by taking aqueous extract at 200 mg/kg and 400 mg/kg body weight. The AEGA and EEGA significantly (p < 0.05, <0.01, <0.001) reduced the number of squares passed by the animals. The study results are shown in the following Fig. 2.

4.3. Rotarod test

In this method, mice falling from the rotarod in both diazepam and AEGA, EEGA treated groups exhibited a reduction in time spent by the mice in the rotarod test compared with the control group. The result was found statistically highly significant (p < 0.05, <0.01, <0.001). The experimental results were shown in Fig. 3.

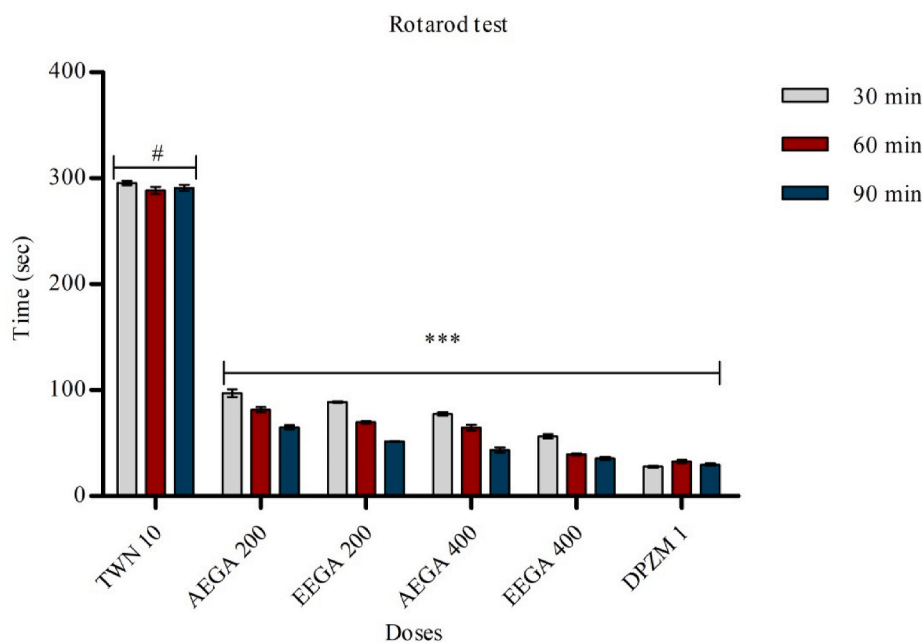


Fig. 3. Effects of test samples in rotarod test. Results are expressed as mean ± SEM (n = 5) and statistically analyzed using ANOVA followed by Dunnett’s comparison test. *p < 0.05, **p < 0.01 ***p < 0.001 have been considered as significant, when compared with control (n = 5). AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

Table 2
Effects of test samples in tail suspension test.

Group	Dose (mg/kg)	Immobility time (sec)
Tween 80	0.1 mL/kg	111.2 ± 2.81
Diazepam	1	212 ± 1.63***
AEGA	200	182 ± 2.65**
AEGA	400	252 ± 3.61***
EEGA	200	198 ± 2.65**
EEGA	400	263 ± 3.35***

Results are expressed as mean ± SEM (n = 5) and statistically analyzed using ANOVA followed by Dunnett's comparison test. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ have been considered as significant, when compared with control (n = 5). AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

Table 3
Effects of test samples in hole cross test.

Group	Dose (mg/kg)	Number of hole crossed in minutes				
		0 min	30 min	60 min	90 min	120 min
Tween 80	10	16.33 ± 1.60	14.67 ± 1.25	15.0 ± 1.00	16.67 ± 0.76	15.00 ± 0.58
Diazepam	1	19.00 ± 0.86***	6.00 ± 1.32***	5.67 ± 1.52***	5.00 ± 1.00***	3.67 ± 0.57***
AEGA	200	16.93 ± 1.11***	4.47 ± 0.88**	4.12 ± 1.11***	3.41 ± 1.70***	5.12 ± 0.78***
AEGA	400	22.45 ± 1.42***	3.97 ± 0.95***	3.10 ± 1.53***	2.43 ± 1.77***	2.89 ± 0.99***
EEGA	200	20.33 ± 1.45***	4.67 ± 1.25***	3.00 ± 1.00***	3.33 ± 1.50***	5.00 ± 1.00***
EEGA	400	20.68 ± 1.89***	3.00 ± 2.17***	2.33 ± 1.25***	2.33 ± 1.25***	3.33 ± 1.25***

Results are expressed as mean ± SEM (n = 5) and statistically analyzed using ANOVA followed by Dunnett's comparison test. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ have been considered as significant, when compared with control (n = 5). AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

4.4. Tail suspension test

The extracts at the dose of 200 mg/kg found a significant ($p < 0.01$) rise in the immobility times when compared to the control group and at the dose of 400 mg/kg found a ($p < 0.001$) rise in the immobility times when compared to control group. In this test, the influence of the extract on behavior is shown in Table 2.

4.5. Hole cross test

The aqueous and absolute ethanol extract of *G. applanatum* was exposed to screen the anti-depressant potential by hole cross method. The result was statistically significant ($p < 0.001$) presented in Table 3.

4.6. Hole board test

The test results are captured in Fig. 4, and it represents the anxiolytic effect of aqueous extract of *G. applanatum*. The extract at the doses of 400 mg/kg has shown most significantly ($p < 0.001$) improved the head dipping numbers compared to control. At the doses of 200 mg/kg, the head dipping number also increased significantly ($p < 0.01$).

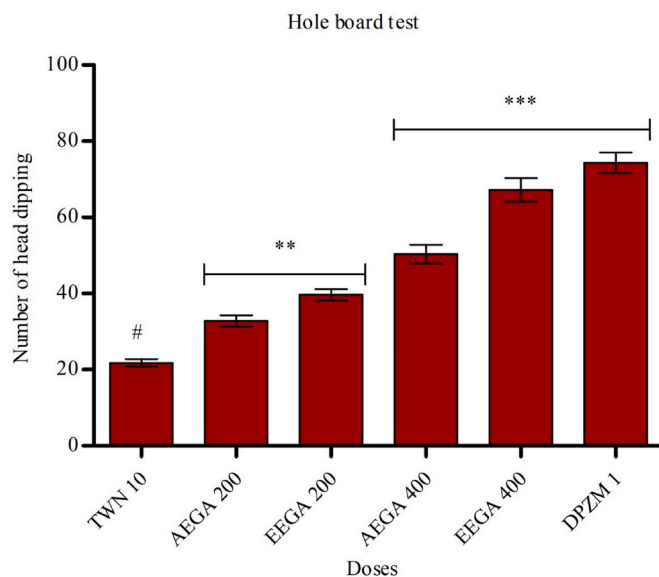


Fig. 4. Effects of test samples in hole board test. Results are expressed as mean ± SEM (n = 5) and statistically analyzed using ANOVA followed by Dunnett's comparison test. * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$ have been considered as significant, when compared with control (n = 5). AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

4.7. Elevated plus-maze test

The extracts of *G. applanatum* significantly decreased the number of entrances of mice into the open arms. The staying time in the open arms of the EPM is presented in Fig. 5. The standard drug (diazepam 1 mg/kg, i. p) found a significant ($p < 0.01$) rise in time spent in open arm and a significant ($p < 0.01$) rise in the number of open arm entries.

4.8. Acetic acid induced writhing

The test was executed by taking aqueous and ethanol extracts (AEGA and EEGA) at doses of 200 mg/kg and 400 mg/kg body weight. The result was found statistically significant. Experimental results are tabulated in Table 4.

4.9. Tail immersion test

The test was performed by taking extract at doses of 200 mg/kg and 400 mg/kg body weight, and the tail withdrawal reflex time represented almost the same as increasing the dose of the sample compared to the standard drug. Highly potential ($p < 0.001$) action was originated by the treatment of AEGA and EEGA at a dose of 200 mg/kg in 60 min, which was $4.38 ± 0.56$, and the administration of AEGA and EEGA at a dose of 400 mg/kg in 60 min and 90 min, which was $4.11 ± 0.07$ and $4.79 ± 0.06$ respectively (Table 5).

4.10. Hot plate test

AEGA and EEGA augmented the latency time at doses of 200 and 400 mg/kg after 60, 120, and 180 min, respectively. At a dose of 400 mg/kg, the extract showed significant ($p < 0.001$) at a time interval of 120 and 180 min (Table 6).

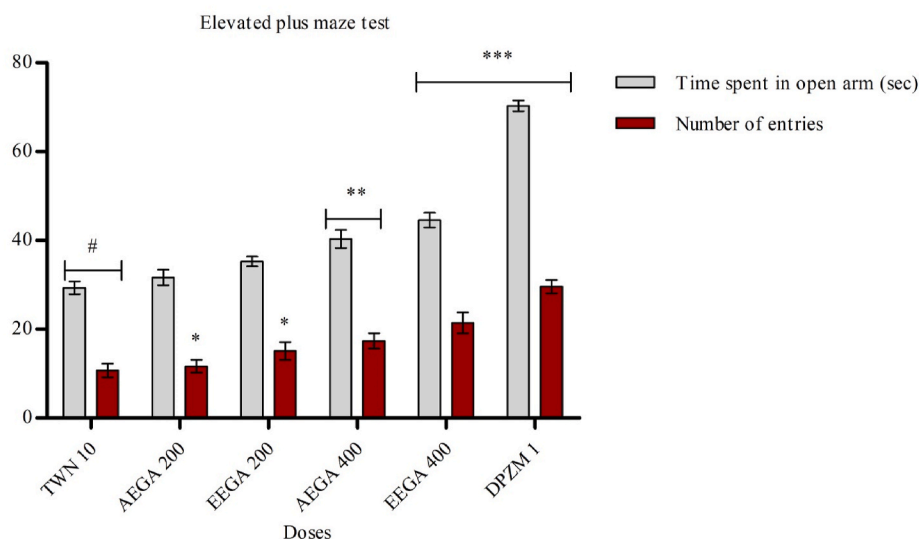


Fig. 5. Effect of test samples in elevated plus-maze test. Results are expressed as mean \pm SEM ($n = 5$) and statistically analyzed using ANOVA followed by Dunnett's comparison test. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ have been considered as significant, when compared with control ($n = 5$). AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

Table 4
Effect of test samples in acetic acid induced writhing test.

Group	Dose (mg/kg)	Writhing count	% of Writhing	% of Inhibition
Tween 80	10 mL/kg	48.8 \pm 1.40	–	–
Diclofenac sodium	10	7.2 \pm 0.35***	15.36	84.63
AEGA	200	39.3 \pm 0.78*	78.55	21.45
AEGA	400	30.5 \pm 0.98*	62.25	37.75
EEGA	200	32.9 \pm 0.87*	67.41	32.58
EEGA	400	27.2 \pm 0.98*	79.09	44.26

Results are expressed as mean \pm SEM ($n = 5$) and statistically analyzed using ANOVA followed by Dunnett's comparison test. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ have been considered as significant, when compared with control. AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

4.11. In silico computer aid virtual screening

In the current review, the maximum and least interaction with Potassium channel KCSA-FAB (PDB: 4UUJ), was demonstrated by ganoapplanin and nigragillin with docking scores of -10.5 kcal/mol and 5.7 kcal/mol respectively, whereas the standard drug Diazepam attained -7.3 kcal/mol binding affinity. By interacting with pro154 residue, Ganoapplanin had interacted with the KCSA-FAB Potassium channel. Table 7 and Fig. 6 displays the relationships of the compounds. Nigragilline has the least docking score (-5.2 kcal/mol) on this simulation of molecular docking with the selected compounds and human serotonin transporter (PDB: 5I6X). Sphaeropsidin D also demonstrated strongest binding affinity (-9.2 kcal/mol). Sphaeropsidin D interacted with the drug pocket via a number of residues (ser203, his236 and his240). In addition, Cytosporone C displayed strongest (-12.8 kcal/mol) binding affinity to the Cyclooxygenase-1 (PDB: 2OYE) receptor where the standard drug showed binding affinity to the same receptor as (-6.9 kcal/mol). Cytosporone C binds to the Cyclooxygenase-1 through a series: conventional hydrogen bond (ser353), pi-sigma (ile523), alkyl bond (val349, met522, trp387, leu384, tyr385, ala527, leu 352) of bonds.

Table 5
Effect of test samples in tail immersion test.

Group	Dose (mg/kg)	Response time (sec)				
		0 min	30 min	60 min	90 min	120 min
Tween 80	10 mL/kg	2.85 \pm 0.02	2.63 \pm 0.03	2.83 \pm 0.15	2.64 \pm 0.03	2.70 \pm 0.09
Diclofenac sodium	10	2.64 \pm 0.03	4.13 \pm 0.08***	5.36 \pm 0.14***	4.38 \pm 0.05***	3.41 \pm 0.11
AEGA	200	2.69 \pm 0.07	3.53 \pm 0.17*	4.38 \pm 0.56***	3.41 \pm 0.02	2.87 \pm 0.09
AEGA	400	2.63 \pm 0.03	3.33 \pm 0.16	4.11 \pm 0.07***	4.79 \pm 0.06**	3.15 \pm 0.08
EEGA	200	2.71 \pm 0.06	3.63 \pm 0.10*	4.21 \pm 0.22**	3.31 \pm 0.05	2.88 \pm 0.07
EEGA	400	2.73 \pm 0.06	3.41 \pm 0.12	4.31 \pm 0.07***	4.99 \pm 0.04***	3.24 \pm 0.07

Results are expressed as mean \pm SEM ($n = 5$) and statistically analyzed using ANOVA followed by Dunnett's test. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ have been considered as significant, when compared with control. AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

5. Discussion

WHO has reported that 80% of people worldwide utilize medicinal plants to treat various diseases due to the presence of phytoconstituents [47]. *G. applanatum* has a wide range of therapeutic activities.

Many mushrooms have been used in previous research to overcome anxiety [48], depressant [49] and pain [50] in different ways. Our study is the first systemic pharmacological and computational evaluation of the efficacy of methanol and aqueous extracts of *Ganoderma applanatum* (MEGA and AEGA) for the treatments of anxiety, depression and so that,

Table 6
Effect of test samples in hot plate test.

Group	Dose	Response time					
		0 min	30 min	60 min	120 min	180 min	240 min
Control	10 mL/kg	8.0 ± 0.71	9.4 ± 0.51	6.8 ± 0.58	8.4 ± 0.51	10 ± 0.71	7.8 ± 0.97
Diclofenac sodium	10	10.6 ± 0.51	14.4 ± 0.43***	12.6 ± 0.51***	13.3 ± 0.68***	8.2 ± 0.86	8 ± 0.89
AEGA	200	9.4 ± 0.51	10.4 ± 1.14	12.2 ± 0.58**	11.00 ± 0.71	14 ± 1.41**	13 ± 0.71**
AEGA	400	8.5 ± 0.71	11.44 ± 1.6	13.54 ± 1.18**	14.7 ± 1.99***	18.28 ± 1.37***	11 ± 1.58
EEGA	200	8.1 ± 0.21	8.5 ± 1.10	11.4 ± 0.40	12.5 ± 0.52	13.5 ± 1.01**	13.4 ± 0.53**
EEGA	400	9.75 ± 0.11	8.1 ± 0.11	12.3 ± 0.35**	13.5 ± 0.41	16.31 ± 0.45***	12.47 ± 0.81**

Results are expressed as mean ± SEM (n = 5) and statistically analyzed using ANOVA followed by Dunnett's test. **p* < 0.05, ***p* < 0.01 ****p* < 0.001 have been considered as significant, when compared with control. AEGA = aqueous extract of *Ganoderma applanatum*. EEAA = ethanol extract of *Ganoderma applanatum*.

Table 7

Docking scores of the selected compounds of *Ganoderma applanatum* mushroom with the potassium channel KCSA-FAB (PDB: 4UJ) and human serotonin transporter (PDB: 5I6X) and Cyclooxygenase-1 (PDB: 2OYE) receptors.

Compounds	PubChem CID	Docking Score (kcal/mol)		
		Anxiolytic	Antidepressant	Analgesic
		4UJ	5I6X	2OYE
Applanatumol E	132542814	-7.1	-6.0	-6.4
Cytosporone C	10778975	-5.7	-5.8	-12.8
Nigragillin	15939563	-5.7	-5.2	-7.3
Ganoapplanin	11067914	-10.5	-6.8	-7.1
Myrocin C	132581256	-7.5	-6.8	-7.9
Sphaeropsidin D	636779	-7.9	-10.5	-6.9
Standard drugs (Diazepam/ Diclofenac sodium)	3016/ 5018304	-7.3	-6.4	-6.9

this investigation was designed to estimate the CNS depressant, anxiolytic, and analgesic activity of AEGA and EEGA.

CNS excitability levels are observed by a reaction, which is known as locomotion. An increase in locomotor activity represents the anxiolytic effect, while a decrease in locomotor activity indicates the sedative effect [51]. Our current study showed that AEGA and EEGA possessed strong CNS depressant potential in the open field, rotarod, tail suspension, and hole cross tests. The extracts' CNS depressant action is assured when the locomotor activity and animals' exploratory behavior are reduced in a dose-dependent manner. Both doses of EEGA and AEGA showed CNS depressant activity in all tests.

The hole board and EPM are the most feasible model for the evaluation of anxiolytic effect. This study also planned to determine of the anxiolytic effect of AEGA and EEGA by using the hole board and EPM test on Swiss albino mice. For the determination of anxiolytic actions of numerous medicinal plant extracts, the hole board is frequently utilized. A high level of anxiolytic behavior is confirmed when head dipping of animals in the hole board increases. On the other hand, a fall in head dipping in the hole board exhibits a high anxiety behavior [52]. In this study, both doses of AEGA and EEGA significantly increased the head dipping in the hole board compared to control. Standard drug diazepam also increased head dipping in the hole board when compared to control. There is a tendency of animals to leave the open area in EPM. This leaving tendency of mice from an open arm reflects the anxiolytic effect. Therefore, an increase in open arm activity of mice expresses an increase in anxiolytic activity [1]. In this study, both doses of AEGA and EEGA significantly decreased both the stay time and the number of entries in open arms when compared with control. In contrast, diazepam increased both the stay time and the number of entries in open arms than control. So, the extracts showed anxiolytic activity only in the hole board method.

Various earlier researches suggested that phytoconstituents like alkaloids, flavonoids, tannins, sterol, saponins, and terpenoids are accountable for CNS depressants anxiolytic effect [1,47,51]. Gamma aminobutyric acid is a chief neurochemical in the CNS. *G. applanatum* showed actions might be due to the enhancement of GABAergic suppression in the CNS via hyperpolarization of membrane. Hyperpolarization might cause a reduction in the rate of firing of some crucial neurons within the brain. *G. applanatum* could also work by direct activation of GABA receptors [53]. Another study also stated that the reduction of serotonin and noradrenaline also plays a part in motor activity. Decrease secretion of serotonin and noradrenaline indicates CNS depressant activity [54]. It is reported that plants containing saponins, tannins, and flavonoids have a beneficial role in numerous CNS disorders [47,55]. The supposed anxiolytic action of the AEGA and EEGA might be due to the binding of plant constituents like flavonoids and saponins to the GABA-A and benzodiazepine complex [56].

The most valuable method for screening the peripheral analgesic effect is the acetic acid-induced writhing test [6]. Endogenous pain mediators, such as prostaglandins, histamine, and bradykinin, are induced when a 0.7% solution of acetic acid is administered intraperitoneally in mice. As a result, pain sensation occurs in a localized area of mice [57]. At the time of the writhing test, the concentration of lipooxygenase products and prostanoids (PGE2 and PGF2α) was significantly raised in the peritoneal fluid [38]. In this study, both doses of AEGA and EEGA showed significant analgesic activity. So the observed analgesic activity of the AEGA and EEGA might be due to its possible interruption with those mentioned pain mediators' actions. A study revealed that alkaloids possess excellent analgesic activity [58]. The phytochemical study of AEGA and EEGA revealed alkaloids, which might act as major contributors to the anti-nociceptive effect.

Tail immersion and hot plate methods are suitable methods for screening centrally acting analgesic effects [59]. According to the procedure used in the tail immersion test, the reaction time of the tail withdrawal reflex in mice will be prolonged if the drugs show morphine-like activity [60]. On the contrary, in the hot plate model, the sensitiveness of animal paws is found at 50–55 ± 1 °C temperatures [40]. The analgesic effect of the drug is increased when the PRT time is increased [61]. Both doses of AEGA and EEGA significantly increased the PRT when compared to control. So, the extracts are believed to have centrally acting analgesic effects. In order to produce new medicines, the development of computer-aided drugs (CADD) plays a key role. Two types of drug design approaches are primarily available: structural and ligand based drug design [62]. In the previous research, researchers used ligand-based relations to pick a lead compound that demonstrated a strong affinity with the potassium channel (PDB: 4UJ) [63], human serotonin receptor (PDB: 5I6X) [32] and COX-1 (PDB: 2OYE) receptors [37,38]. The MAO receptor is used in this study because MAO-A is typically aimed at treating depression and anxieties [64]. In this

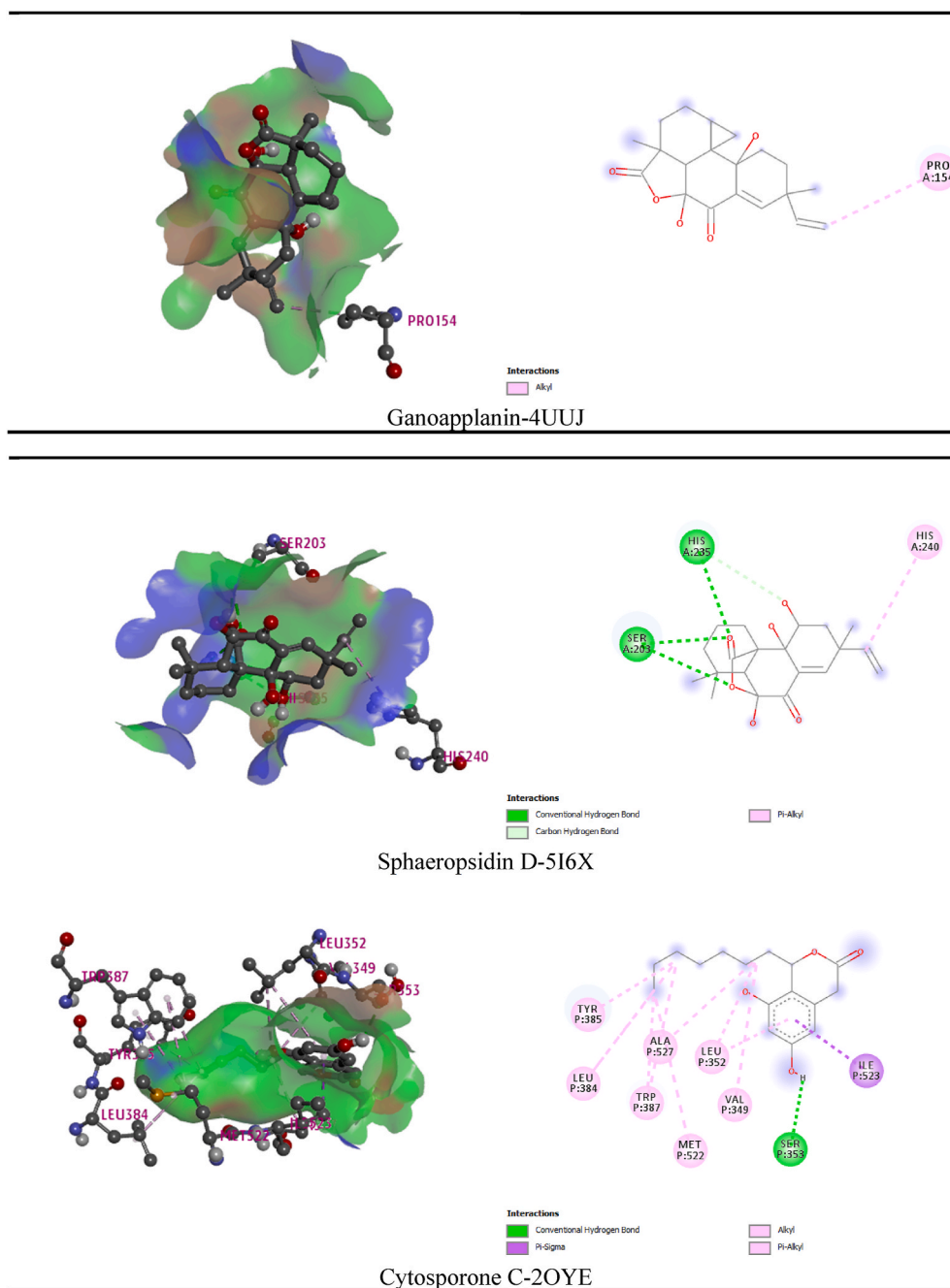


Fig. 6. Best interaction (3D and 2D) of the selected compounds of *Ganoderma applanatum* mushroom with the Potassium channel KCSA-FAB (PDB: 4UUJ) and human serotonin transporter (PDB: 516X) and Cyclooxygenase-1 (PDB: 2OYE) receptors.

research, molecular docking was carried out for six main compounds of *G. applanatum* with the active site of the enzymes: 4UUJ, 516X and 2OYE. The analysis demonstrated that, Ganoapplanin, Sphaeropsidin D and Cytosporone C have the best docking score of -10.5 , -10.5 and -12.8 with the 4UUJ, 516X and 2OYE enzymes correspondingly, which also recommends that they have a superior interface with the 4UUJ, 516X and 2OYE receptors than the other bioactive constituents. However, more experiments to confirm the pharmacological implications of these isolated substances are required.

6. Conclusion

Our analysis exhibited that the AEGA and EEGA have substantial medicinal property via its CNS depressant, anxiolytic, and analgesic

activity, all of the activity measured with the standard reference drug. This study suggests that the biological constituents present in the plant are worthy of further investigation and structure elucidation.

Author's contribution

SMMH: conceptualization, research design, acquisition, supervision. MJJ: investigation, formal analysis, data curation. MSH: formal analysis, data curation, wrote the manuscript. MR: formal analysis, data curation. AB: formal analysis, data curation. MGU: visualization, validation, review and editing. NUE: methodology, investigation, research design, software, writing the original manuscript, review and editing. Finally all the authors reviewed and agreed to publish this research.

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Conflicts of interest

The authors have no known competing interests.

Ethical consideration

In keeping with the ethical principles set forth in the Helsinki 2013 Declaration [65,66] all biological activity evaluation was carried out.

Declaration of competing interest

The authors declare no conflict of interest for this research.

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