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Original article

Neuropharmacological and antiproliferative activity of *Tetrastigma leucostaphyllum* (Dennst.) Alston: Evidence from *in-vivo*, *in-vitro* and *in-silico* approaches

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ABSTRACT

As the incidence of neurodegeneration and cancer fatalities remains high, researchers are focusing their efforts on discovering and developing effective medications, especially plant-based drugs, against these diseases. Hence, this research aimed to investigate the neuropharmacological potentials of aerial parts of Tetrastigma leucostaphyllum, employing some behavioral models, while the antiproliferative effect was explored against a panel of cancer cell lines (MGC-803, A549, U-251, HeLa and MCF-7) using a colorimetric assay. In addition, active extracts were analyzed by GC–MS technique to identify the active compounds, where some selective compounds were docked with the particular pure proteins to check their binding affinity. Results from neuropharmacological research indicated that the total extract and its fractions may be effective ($p = 0.05$, 0.01, and 0.001, respectively) at doses of 100, 200, and 400 mg/kg of animal body weight. The greatest antidepressant and anxiolytic effects were found in the *n*-hexane fraction. The n-haxane fraction also exhibited the highest cytotoxicity against the U-251 cell line (IC5014.3 µg/mL), followed by the A549, MG-803, HeLa, and MCF-7 cell lines, respectively. From the *n*-hexane fraction, ten chemicals were detected using the GC–MS method. Additionally, the in-silico research revealed interactions between the *n*-hexane fractions' identified compounds and the antidepressant, anxiolytic, and cytotoxic receptors. The molecules showed binding affinities that ranged from 4.6 kcal/mol to 6.8 kcal/mol, which indicates the likelihood that they would make good drug candidates. This study highlighted the plant's neuropharmacological and cytotoxic properties, however, more research is needed to determine the etymological origin of these effects.

1. Introduction

Anxiety is the disordered version of normal fear. It causes changes in mood, thoughts, behavior, and how the body works (Pérez-Edgar and Fox, 2005).Because of how common it is and how much it affects people, it has become an important area of research in psychopharmacology (Goyal and Sasmal, 2014).Even though scientists don't fully understand where most anxiety disorders come from (Pérez-Edgar and Fox, 2005), this is now a topic of interest.Also, one in four people have mental or neurological diseases, and 264 million people have depression (Khan et al., 2022).Globally, 60.8% of people have moderate to severe depression, 73% have anxiety, and 62.4% have stress (Kulsoom and Afsar, 2015). Therefore, 20% of people in the world have been

Abbreviations: CNS, Central nervous system; GC-MS, Gas chromatography-mass spectroscopy; VCF, Village common forest; NTL, *n*-hexen Extract of *Tetrastigma leucostaphyllum*; BTL, Butanol Extract of *Tetrastigma leucostaphyllum*; DTL, Dichloromethane Extract of *Tetrastigma leucostaphyllum*; MTL, Aqueous Extract of *Tetrastigma leucostaphyllum*; TST, Tail suspension test; FST, Force Swimming Test; EPM, Elevated plus-maze test; OFT, Open field test; HCT, Hole Cross Test; TIC, Total Ionic chromatogram.

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affected by depression, which is a crippling and potentially fatal illness (Manji et al., 2001; Charney, 2004). In Bangladesh, anxiety, stress, and depression levels have been reported to be as high as 64.8%, 59.0%, and 54.3%, respectively (Mamun et al., 2021; Arusha and Biswas, 2020). Almost 7 million people in Bangladesh experience depressive and anxiety disorders, respectively. It is also unclear what causes the remainder of the non-genetic risk. Some suggest that early childhood trauma, mental stress, physical disease, and even viral infections may be responsible for depression. In recent years, several powerful antidepressants have been introduced. For example, selective serotonin reuptake inhibitors (SSRIs), neural response imaging (NRI), and dual serotonin/norepinephrine reuptake inhibitors (SNRIs) are popular antidepressants, and existing medicines with distinct pharmacology are also commonly prescribed as antidepressants (Khushboo and Sharma, 2017). Despite the lack of definitive data showing that antidepressant medicines are less potent in treating depression, therefore, plant-based antidepressants need to be explored, which are considered safer, more affordable, and produce less toxicity, increasing the possibility that they may be beneficial in other mental diseases (Khushboo and Sharma, 2017; Kaur and Kumar, 2012; Andrews and Pinder, 2001).

On the other hand, cancer is a tumor that develops from an abnormal growth of cells that begins locally but eventually spreads across the body. It is the second greatest cause of mortality on the globe, accounting for 9.6 million fatalities in 2018 and one out of every six deaths that occur on our planet. According to the WHO, around 70% of cancer-related fatalities take place in countries with low and moderate incomes (Amin et al., 2020). In Bangladesh, cancer is the sixth most prevalent cause of death, and 60 percent of cancer patients die within five years of being diagnosed with the disease (Alam et al., 2020). To treat cancer, chemotherapy is one of the four basic options besides surgery, radiation, and immunotherapy, and there is a range of cancer-targeting chemotherapeutics available (Cassidy and Setzer, 2010). However, because of the harmful effects of synthetic chemical entities, the preference for herbal items over synthetic treatments is growing at a high rate. Extensive studies are required to see whether indigenous plants could be utilized to cure diseases like cancer and infectious diseases in humans (Kausar et al., 2022). In Bangladesh, for the first time, a total of 1479 medicinal plants were recorded by our research group (Uddin et al., 2010). However, only a small portion of these recorded species were studied for their biological and phytochemical properties. Taken together, this study selected one ethnomedicinal plant, Tetrastigma leucostaphyllum (Dennst.) Alston (family - Vitaeae), also known as Horina lata, which is a plant with many therapeutic qualities. For example, a paste of leaves and roots is applied to the head to treat fever, gout, oedema, diarrhea, stomach disorders, stomachaches, and a pill made from the root is used to enhance growth in children (Rudra et al., 2020a). Among these uses of this plant, only the antidiarrheal properties of the plant extracts were explored in our previous studies (Rudra et al., 2020b). Moreover, in Bangladesh, traditional healers of indigenous communities use the leaves and roots of Tetrastima leucostaphyllum (T. leucostaphyllum) to obtain medicine for cancer and neurological disorders. For example, paste prepared from the leaves and stem is used by the Marma people to reduce the size of the tumor, while the Tripura community takes leaf juice three times daily for one month to cure tumors. Likewise, Chakma communities prepare pills using the leaves and stems of this plant and use them against neurological disorders (personal communication). On the other hand, some other species of this genus Tetrastigma are used as antiproliferative, antiinflammatory, antiviral, antioxidative, hepatoprotective, and to treat neurological disorders in China (Zhang et al., 2022). However, no systematic research has been conducted to date on the effects of the studied plant on central nervous system (CNS) activity and cytotoxicity. Considering these, we performed in vitro and in vivo assays to discover the neuropharmacological potential and antiproliferative effect of the chosen plant. In addition, gas chromatography-mass spectroscopy (GC–MS) was implemented to detect bioactive constituents of the active fraction n-hexane. An in silico computer-aided screening method (molecular docking) was employed to explore lead compounds with possible mechanisms of action to treat mental illness and cytotoxic effects.

2. Materials and method

2.1. Drugs and chemicals

Thiopental sodium and Diazepam were purchased from Square Pharmaceuticals Ltd., Bangladesh. Methanol, n-hexane, dichloromethane, cisplastin, and tween 80 were brought from Merck Life Sciences Private Limited (Bengaluru, Karnataka, India), and analytically graded reagents were used for the biological investigations.

2.2. Collection and identification of the plant

From October to November 2016, aerial parts of T. leucostaphyllum were taken from the Beganasori VCF in Rangamati, Bangladesh. Special precautions were taken to prevent adulteration, and only healthy and disease-free materials were obtained for the experiment. The studied plant was identified by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chattogram-4331, Bangladesh, and a voucher specimen was deposited and preserved as a reference for further repetition of the experiment in the herbarium of the University of Chittagong with accession number (CTGUH SR7912).

2.3. Extraction and fractionation

Research-grade standard aerial parts were dried and crushed into powder using an electric blender. 570 g of powder were soaked in 10 L methanol and kept at room temperature for four days with regular stirring and shaking. Then a crude extract of methanol was obtained by filtering through a cotton plug and Whatman No. 1 filter paper. The same procedure was repeated three times, and the methanol extract yielded 80.5 g. Finally, part of the crude extract of methanol (30 g) was fractionated into n-hexane (NTL), dichloro-methane (DTL), and butanol (BTL) following the Kupchan partitioning method (Rahman et al., 2016). Each fraction was reduced using a rotary evaporator to obtain 10.35, 8.58 and 4.88 g, respectively.

2.4. Investigations

2.4.1. Experimental animals

Healthy and certified mice (weighing 25–30 g) were purchased from Jahangirnagar University, Bangladesh [BBECJU/M2018(3)1]. Purchased mice were quarantined in standard conditions and observed for a week. A normal laboratory diet and distilled water (*ad libitum*) were provided to the mice before starting the experiments. They were kept on a natural day-night cycle with adequate ventilation throughout the experiment. The tests were carried out in a completely isolated and noiseless environment. The animals were brought into the laboratory and acclimated for 15 days before the test. The research protocol was approved by Jahangir Nagar University, Savar, Bangladesh [BBECJU/M2018(3)1].

2.4.2. Experimental design

In all models, animals were indiscriminately separated into one hundred and two groups: acute toxicity (4 groups), positive control (7 groups), negative control (7 groups), and seven experimental groups (3 doses × 4 samples × 7 experimental models = 84 groups). Each group consisted of six animals (except for acute toxicity), and crude drugs were administered at three typical doses (100, 200, and 400 mg/kg; b.w; p.o.). The vehicle (1 % tween 80, 10 mL/kg,b.w; p.o.) was used as negative controls, while Diazepam (2 mg/kg; b.w; i.p.) used as positive controls in all models.

2.4.3. Acute oral toxicity test

A group of five swiss albino mice were subjected to an acute oral toxicity test in accordance with the OECD 425 (Toxicity—Up, 2001) guideline and treated with a single oral limit dose of 2000 mg/kg b.w. of each fraction of plant extract. Five female Swiss albino mice were chosen randomly for the experiment. After being dosed at the maximum test dose, one animal was given a 24-hour follow-up and survived. Following the successful survival of the test animal, the rest of the animals were given 2000 mg/kg doses in a predefined sequence for a total of five animals to be evaluated using this method. Each of the treated animals was examined once in the first 30 min of treatment, eight times in the first 24 h at three-hour intervals, and at least once a day for the next 13 days for any indications of toxicity.

2.5. Assessments of antidepressant activity

2.5.1. Tail suspension test (TST)

In this study, Stukalin et al.'s (Stukalin et al., 2020) latest method was used to figure out how long a fish would stay still after having its tail suspended. In short, the mice were hung about 1 cm from the end of their tails about 50 cm above the ground using sticky tape. During the 6-minute test, the amount of time spent immobile and the number of times efforts were made to escape discomfort were recorded.

2.5.2. Force swimming test (FST)

The FST was carried out, followed by the previously described method (Kulesskaya and Voikar, 2014). After thirty minutes of the administration of T. leucostaphyllum extracts (MTL, BTL, NTL, and DTL) at a concentration of 100–400 mg/kg/b.w., standard drug (diazepam 2 mg/kg), and vehicle (10 mL/kg), the mice were kept for six minutes in a custom-made, standard-sized (35 cm height × 24 cm diameter) cylindrical container filled with a certain level of water (13.5 cm) at room temperature. The first minute was considered a trial session, and the last 5 min were a test session to assess the immobility of the treated mice. Mice were floated with minimum motion, keeping themselves in submerged conditions by just raising their heads above the water. The duration of immobility was extended and interpreted as evidence of antidepressant efficacy.

2.6. Assessments of anxiolytic activity

2.6.1. Elevated plus-maze test (EPM)

The EPM test has been validated to assess anxiety in mouse models (Emon et al., 2020). The plus-maze was composed of two open (30 × 5 × 25 cm) and two covered (30 × 5 × 25 cm) arms. All the arms were led from a shared center platform (5 × 5 cm). The wooden plus-maze was placed on a surface 45 cm above the ground. An edge (0.25 cm) was added to encourage mice to explore around the perimeter of the enclosure. Before the thirty minutes of administering the crude extracts (MTL, BTL, NTL, and DTL at a concentration of 100–400 mg/kg b.w.) and other test samples, each animal was released in the center of the plus-maze. The number of entrances as well as the time spent by each mouse in the open and covered arms were monitored and noted (Emon et al., 2020; Hossen et al., 2021). An ethanol solution was used to clean the maze as an antiseptic after it was subjected to the test for each animal.

2.6.2. Hole-Board test

The hole board test was carried out on a wooden floorboard of standard size (40 cm × 40 cm × 25 cm) with uniform holes. In this experiment, the animals were placed separately in the center of a hole board. The total number of head dips from the hole of the board by the experimenting mice within five minutes was investigated and recorded (Arenas et al., 2014).

2.7. Assessments of sedative properties

2.7.1. Open field test (OFT)

The protocol described by Sáenz et al. (Sáenz et al., 2006) was followed to carry out the open field test. In the experimental setup, the floor of an open field was divided into some squares, each of which has been alternately painted in black and white to create a pattern. The apparatus has a 40-centimeter-high wall around it. Before the experiment, the *T. leucostaphyllum* extracts (MTL, BTL, NTL, and DTL) were administered to the mice in three different dosages (100, 200, and 400 mg/kg; p.o.), while the mice in the negative control group were given Tween 80 (10 mL/kg, p.o.), and the standard group was given Diazepam (2 mg/kg, i.p.). Then, treated mice were released in the center of the open field. A three-minute count was then made to count the total number of squares visited by each treated mouse at the following periods: 0, 30, 60, 90, and 120 min after administering standard (i.p.) and test samples (p.o.).

2.7.2. Hole Cross test (HCT)

A previously established method (Arenas et al., 2014) was used to conduct this test. A divider was installed inside a cage that measured 30 × 20 × 14 cm. The height of the case was 7.5 cm, and a hole was drilled in the middle of the cage with a three-centimeter diameter. Then mice were treated with the test samples and placed in a cell along the side of a chamber. The total number of mice passing from one chamber to another through the hole was counted for 3 min at the following time intervals: 0, 30, 60, 90, and 120 min after administering test samples and control drugs.

2.7.3. Thiopental sodium-induced sleeping time test

An established protocol (Ali et al., 2015) was followed to implement this test. In this experiment, extracts (MTL, BTL, NTL, and DTL at a concentration of 100–400 mg/kg; b.w.), and control doses were administered orally, while the standard drug Diazepam (2 mg/kg) was administered intraperitoneally. After 30 min, sleep-inducing drugs, thiopental sodium (40 mg/kg, i.p.) was injected intraperitoneally into each mouse. The animals ' behavior was measured as the latent period (interval between the injection of thiopental sodium and lack of righting reflex) and the sleeping period (interval between the loss and recovery of reflex).

2.8. Cytotoxicity evaluation on cells (MTTs) by tetrazolium bromide assay

The MTT colorimetric method was used to test the cytotoxicity of extracts (Sharma et al., 2009).Briefly, cell cultures were collected, and approximately 5 × 103 cells (cell line) were introduced per well of flat-bottomed 96-well plates (Becton Dickinson, Cockeysville, MD, USA). Seeded plates with 100 μL of Dulbecco's Modified Eagle Medium (DMEM) were incubated. The medium was replaced by fresh medium after 24 h, and the plant extract at seven

different concentrations (800 μg/mL; 400 μg/mL; 200 μg/mL; 100 μg/mL; 50 μg/mL; 25 μg/mL and 12.5 μg/mL) was seeded. As controls, we used the antitumor drug Cisplatin (CDDP) (positive) and culture medium (negative). After that, the medium was replaced by adding 100 μL fresh medium, which contained 10 μL MTT (5 mg/mL). Finally, the medium was replaced by adding 200 μL of DMSO to dissolve the formazan crystals. All the cell cultures, including both controls, were incubated at 37 °C in a 5% CO_2 incubator. The exact background noise measurement was observed using a microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT, USA) to determine the absorbance at 570 nm and 630 nm wavelengths. The optical density background at 630 nm was subtracted from the total optical density signals at 540 nm.

2.9. Gas Chromatography/Mass spectroscopy

The chromatography of n-hexane fractions of T. leucostaphylum was executed using a Shimadzu gas chromatography-mass spectrometer (Shimadzu GC–MS QP2010PLUS, Shimadzu Corporation, nakagyo-ku, Japan) and a DB-5MS capillary column (30 m × 0.25 mm × 0.25 mm). Our prior research (Faruque et al., 2019) provided a detailed description of the methods.

2.10. In silico molecular analysis

2.10.1. Molecular Analysis: Software tools

UCSF Chimera, AutodockVina, Discovery Studio Visualizer 2020 (BIOVIA), Protein Data Bank (PDB), MGL instruments, the Protein Data Bank (PDB), and PubChem were employed.

2.10.2. Molecular analysis: Selection of target proteins

It was chosen mostly through a systematic review of the scientific research that has already been done to find possible therapeutic targets for depression and anxiety. The chosen targets were verified using the protein data library (PDB; https://www.rcsb.org/; extensive biological molecules database, viz.) (Goodsell et al., 2020).

2.10.3. Molecular analysis: Preparation of target proteins

Potassium channels control how potassium ions move across the cell membrane, which affects the cell's electrical potential. The resting membrane potential and action potential are important parts of how cells control their size and how many of them they make. This makes them important targets for neurological disorders. In addition, corticotropin-releasing factor, or CRF, is a hormone that is made by the hypothalamus and released when the body is stressed. CRF causes the anterior pituitary gland to make more adrenocorticotropic hormone (ACTH), which in turn makes the adrenal glands make more cortisol. Cortisol is a hormone that helps control how the body reacts to stress and is involved in many bodily functions, such as the way glucose is used, how the immune system works, and how blood pressure is controlled. Besides, the human serotonin transporter (SERT) is a membrane protein that is expressed on the presynaptic neuron and is responsible for the reuptake of serotonin, a neurotransmitter, into the presynaptic neuron. This reuptake process terminates the action of serotonin, allowing for the regulation of serotonin levels in the synaptic cleft and ensuring proper neurotransmission. SERT is the target of many antidepressant medications, including selective serotonin reuptake inhibitors (SSRIs), which work by blocking the reuptake of serotonin, thereby increasing the concentration of serotonin in the synaptic cleft and enhancing neurotransmission. Cytotoxicity is the ability of a substance to kill cells. Cytochrome P450 2C9 has been linked to cytotoxicity (CYP2C9). This is because CYP2C9 is involved in the way that many substances, like some medicines and toxins, are broken down in the body. Therefore, considering these facts, potassium channel KCSA-FAB (PDB: 4UUJ) (Lenaeus et al., 2014), human corticotrophin-releasing factor (PDB: 4K5Y) (Hollenstein et al., 2013), human serotonin transporter (PDB: 5I6X) (Coleman et al., 2016), human serotonin transporter (PBD: 6VRH) (Coleman et al., 2020), and human cytochrome P450 CYP2C9 (PDB: 1OG5) (Moazzem Hossen et al., 2021) were obtained from the RCSB Protein Data Bank (PDB). These elements have been discovered in humans like; Homo sapiens, Mus musculus, and Escherichia virus T4. Additionally, similar structures were also revealed in current research, including citations from well-recognized journals. Receptors, i.e.; 4UUJ, 4K5Y, 5I6X, 6VRH, and 1OG5, are activated for anxiety, depression, and cytotoxicity. To begin the investigation, the protein ligands have been separated from their complexes using the Discovery Studio Visualizer software (BIOVIA). UCSF Chimera was applied to optimize and prepare these proteins before their binding in the docking phase. The protein structure was completed by removing water molecules from the structures and introducing polar hydrogen to the H-atoms. After estimating the gasteiger charge, the proteins were converted and exported in the pdbqt format using the AutodockVina docking software (Masters et al., 2020).

2.10.4. Molecular analysis: Virtual filtering

It was essential to execute the PyRx-autodock Vina software in order to finish the virtual screening (Masters et al., 2020). The ligands (9,12,15-octadecatrienoic acid, 9-octadecenal, 13-docosenoic acid, 13-tetradecynoic acid, methyl palmitate, methyl linoleate, 6,10,14-trimethylpentadecan-2-one, phytol, and phytyl acetate) of T. leucostaphyllum plant were minimized and optimized by PyRX-autodock vina software and docked with the selected receptors (4UUJ, 4K5Y, 5I6X, 6VRH, and 1OG5). The execution of PyRx autodocking has covered the Ligand-centered grid box binding site. The placement of the grid box allows the ligand to move freely inside the assigned values, passing over and around any of the grid boxes (X, Y, and Z). Applying the corresponding PDB crystals 4UUJ, 4K5Y, 5I6X, 6VRH, and 1OG5, the coordinates of x, y, and z were retrieved using the Discovery Studio Visualizer. As in earlier articles, the grid scale was kept 60 Å x 60 Å x 60 Å. The ligand–protein interactions were displayed using the BIOVIA Discovery Studio Visualizer 2020 software.

3. Results

3.1. Acute toxicity test

The animals had unaltered behavioral, neurological, and autonomous profiles during this test period. At the maximum dosage of extracts, no mortality in mice was detected.

3.2. Antidepressant profiling

3.2.1. Tail suspension test and force swimming test

In mice, a substantial impact on the TST and FST immobility periods was observed after administering the extracts MTL, BTL, NTL, and DTL (100–400 mg/kg; b.w.). The highest doses of crude extract (200 and 400 mg/kg) have substantially reduced immobility ($p < 0.01, 0.001$). In both TST and FST, there was a significant difference between the control group and the test samples, where the NTL fraction yielded the best reduction of mobility in the TST and FST models (Fig. 1).

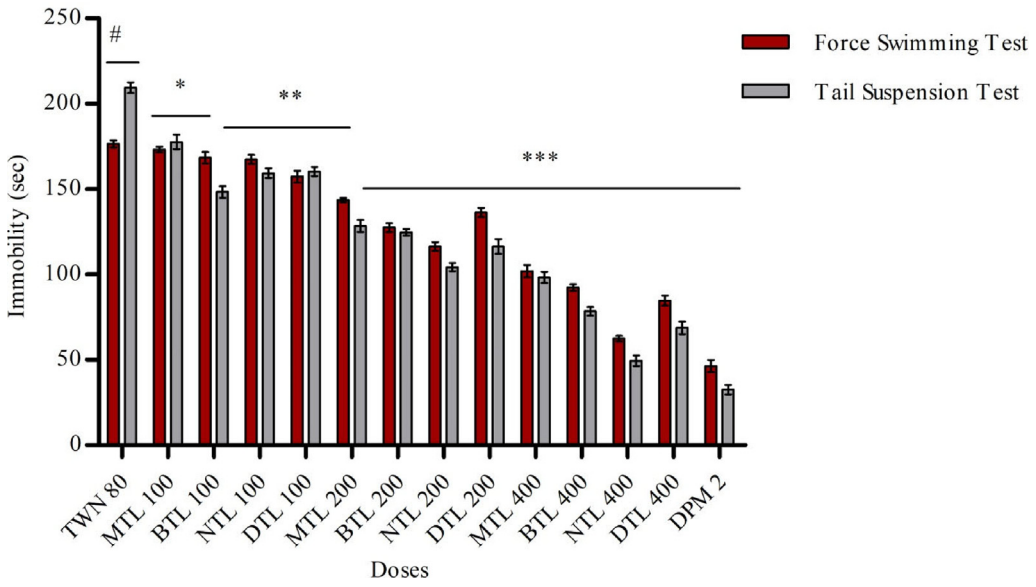

Fig. 1. Effect of n-hexane fraction (T. leucostaphylum) and other fractions on tail suspension test and force swimming test in mice. *p < 0.05, **p < 0.01, ***p < 0.001 were considered significant compared to the control sample in the One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test. NTL, DTL, BTL, and MTL = *n*-hexane fraction of T. leucostaphylum, dichloromethane fraction of T. leucostaphylum, n-butanol fraction of T. leucostaphylum, aqueous extracts of T. leucostaphylum respectively.

3.3. Anxiolytic profiling

3.3.1. Elevated plus-maze test (EPM)

MET and its fraction showed significant activities in the percentage of time spent on open arms at the concentrations of 200 and 400 mg/kg. Administration of diazepam substantially enhanced the number of entries in the open arms. Although the effects of diazepam were more apparent than those of MET and its fractions, the frequency of open arm entries significantly increased (p < 0.05, 0.01, 0.001) (Table 1).

3.3.2. Hole board test

Each of the experimental mice was placed in the hole-board apparatus, and the total number of head pokes by each mouse was recorded. For vehicles, no significant increases in the number of head dips were seen; nevertheless, the rest of the test samples had substantial (p < 0.05, 0.01, 0.001) numbers of head pokes. BTL and NTL fractions at the highest concentration (400 mg/kg b. w.) yielded head pokes of 56.67 ± 1.14 and 60.17 ± 2.23 respectively, while MTL and DTL also raised the number of head pokes substantially (p < 0.01)e compared to control (Fig. 2).

Table 1
Effect of n-hexane and other fractions of T. leucostaphylum leaves with the stem on an elevated plus-maze test (EPM) in mice.

Treatment	Dose (mg/kg,p.o)	Number of entries in the open arm	Time spent in open arm (sec)
Control	10 mg/mL	17.50 ± 1.96$^{\#}$	78.83 ± 0.60$^{\#}$
Diazepam	2	38.83 ± 0.40***	142.12 ± 0.58***
MTL	400	32.45 ± 0.43***	128.43 ± 1.25***
BTL	400	34.26 ± 0.31***	133.48 ± 1.05***
NTL	400	31.26 ± 0.48***	137.63 ± 1.10***
DTL	400	30.48 ± 0.30***	129.36 ± 1.90***
MTL	200	23.56 ± 0.42**	113.27 ± 0.76***
BTL	200	27.17 ± 0.60***	125.17 ± 1.24***
NTL	200	28.72 ± 0.56***	121.43 ± 1.91***
DTL	200	24.23 ± 0.31**	107.23 ± 1.36***
MTL	100	21.38 ± 0.12*	87.33 ± 0.91*
BTL	100	20.20 ± 0.48*	91.10 ± 0.91**
NTL	100	22.83 ± 0.60*	84.57 ± 1.47
DTL	100	20.57 ± 0.43	81.83 ± 2.24

$^{*}p$ < 0.05, $^{**}p$ < 0.01, $^{***}p$ < 0.001 were considered significant compared to the control sample in the One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

3.4. Sedative profiling

3.4.1. Open field test

Locomotion frequency was assessed for 0–120 min in the open field test. Administration of extracts significantly (p < 0.001, 0.01, 0.05) reduced the number of locomotions. However, among the tested extracts, the NTL fraction accounted for the highest retardation of locomotion in mice. After the administration of NTL 400 mg/kg, the locomotion time was 21.77 ± 2.32 in 0 min, and it was decreased to 9.95 ± 0.26 in 30 min, 10.73 ± 2.23 in 60 min, and 8.25 ± 2.40 in 90 min, and 8.70 ± 2.35 in 120 min, respectively, compared to the standard drug. (Table 2).

3.4.2. Hole Cross test

In this test, the crude extracts and their fractions substantially reduced the number of squares moved by mice, both dose-dependently and time-dependently. The most excellent suppression of the squares traveled by the mice was observed with the treatment of all tested fractions at the highest concentration (400 mg/kg, b.w., p.o.), which are summarized in Table 3.

3.4.3. Thiopental sodium-induced hypnotic test

The onset and duration of sleep were evaluated in mice by the thiopental sodium-induced sleep test. A moderate dose-dependent hypnotic effect was seen in crude samples. NTL (400 mg/kg) had the greatest (p < 0.001) hypnotic potential among the test substances. The results were distinguished by comparison with the control group (Fig. 3).

3.5. Cytotoxicity analysis on MGC803, A549, HeLa, U-251, and MCF-7

Based on the traditional usage of the studied plant against tumor/cancer used by the indigenous communities of Bangladesh, we also aimed to perform an MTT assay of this plant against a

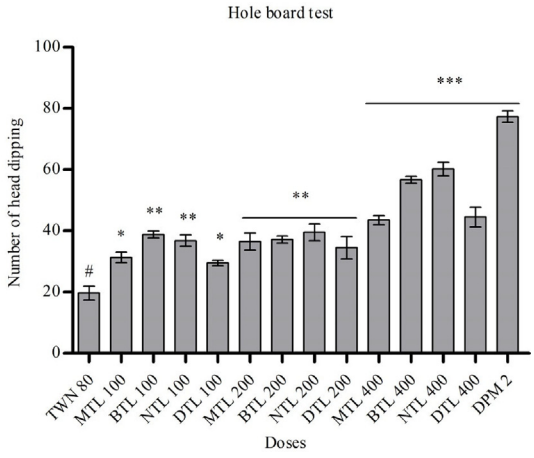

Fig. 2. Effect of *n*-hexane and other fractions (*T. leucostaphylum*) on hole board test in mice. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (n = 6) were considered significant compared to the control sample in the One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

panel of cancer cell lines such as human gastric cancer cells (MGC803), adenocarcinomic human alveolar basal epithelial cells (A549), cervical cancer cells (HeLa), human glioblastoma cells (U-251) and human breast cancer cells (MCF-7) to evaluate the cytotoxicity of plant extracts at seven different concentrations (800 μg/mL; 400 μg/mL; 200 μg/mL; 100 μg/mL; 50 μg/mL; 25 μg/mL and 12.5 μg/mL). To date, these 'lead' species have been evaluated for cytotoxicity in vitro for the first time. MTT assays of extracts have yielded promising results on a panel of cancer cell lines. All tested plant extracts were considered to show the highest cytotoxic activity (>80% inhibition) at the higher concentrations, while lower cytotoxic effects were observed at lower concentrations (Supplementary Figs. 1-5**).** Among all fractions, n-hexane extract showed the highest cytotoxicity against U-251 cells with the lowest IC_{50} value (14.3 μg/ml) (Supplementary Fig. 4**,** Table 4) followed by A549 cells (47.35 μg/mL), MG-803 cells (77.03 μg/ml), HeLa cells (269.6 μg/ml) and MCF-7 cells (478.5 μg/mL), respectively (Table 4**,** Supplementary Figs. 1-5). Likewise, fraction DCM exhibited moderate to low cytotoxic effects against the tested cell lines with IC_{50} values ranging from 92.17 μg/mL to 437 μg/mL while the moderate effect was postulated against the MCF-7 cell line and the lowest effect against the HeLa cell line (Table 4**,** Supplementary Figs. 3 and 5). On the other hand, among all tested fractions, n-butanol showed the lowest inhibition against the tested cell lines, while IC_{50} values were not detectable against MGC 803 and A549 cell lines (Table 4). Furthermore, the aqueous extract of the studied plant had IC_{50} values of 179.3, 276.1, 377.5, 681, and 786.5 μg/mL against MCF-7, 803, A549, HeLa, and U-251 cell lines, respectively (Table 4).

3.6. Gas Chromatography/Mass spectroscopy

The GC–MS analysis of the *n*-hexane fraction (NTL) showed that the retention times of ten compounds ranged from 32.208 to 40.506 min. The chemical compositions of the compounds have been listed in Table 5, the total ionic chromatogram (TIC) is displayed in Supplementary figures 6, and the structure of the identified compounds are displayed in Fig. 4. According to detected compounds, the relative peaks area were as follows: Phytyl acetate (2.96%), 2-pentadecanone, 6,10,14-trimethyl (0.92%), phytol (0.74%), 13-docosenoic acid, methyl ester (1.90%), hexadecanoic acid methyl ester (23.65%), methyl palmitic acid (10.10%), linoleic acid methyl ester (17.93%), methyl elaidolinolenate (29.25%), 13-tetradecynoic acid (7.88%), and 9,12,15-octadecatrienoic acid (4.67%).

3.7. Molecular docking analysis

An effort to dock against analytical proteins in which all the compounds sought a better orientation and conjugation that may interfere with the target protein. Entirely nine (9,12,15-octadecatrienoic acid, 9-octadecenal, 13-docosenoic acid, 13-tetradecynoic acid, methyl palmitate, methyl linoleate, 6,10,14-trimethylpentadecan-2-one, phytol, and phytyl acetate) bioactive constituents have been uncovered for the docking against potassium channel KCSA-FAB (PDB: 4UUJ), human corticotrophin-releasing factor (PDB: 4K5Y), human serotonin transporter (PDB: 5I6X), human serotonin transporter (PBD: 6VRH) and human cytochrome P450 CYP2C9 (PDB: 1OG5) for the antidepressant, anxiolytic, sedate-hypnotic and cytotoxic activities, respectively. Initially, based on the scoring function, all compounds were screened. The glide score of Phytyl acetate was almost the highest for both 4UUJ and 4K5Y proteins. The phytyl acetate interacted with gly42, val93, pro154, pro41, gln39, and asn41 residues of the potassium channel enzyme. Phytyl acetate also reacted with pro241, phe240, pro240, phe240, and val244 residues of the human corticotrophin-releasing factor enzyme and obtained the highest binding affinity among the other phytoconstituents. The

Table 2
Effect of extracts of *T. leucostaphylum* on open field test (OFT) in mice.

Treatment	Dose (mg/kg, p.o.)	Locomotion frequency				
		0 min	30 min	60 min	90 min	120 min
TWN	10 mg/mL	23.83 ± 1.67$^{\#}$	28.33 ± 2.09$^{\#}$	26.50 ± 1.56$^{\#}$	22.33 ± 1.67$^{\#}$	20.67 ± 1.68$^{\#}$
DPM	2	22.83 ± 2.13	6.50 ± 0.62***	5.17 ± 2.70***	3.33 ± 2.42***	3.83 ± 0.79***
MTL	400	23.15 ± 2.50	10.40 ± 1.43***	9.55 ± 1.03***	7.32 ± 3.43***	8.45 ± 2.34***
BTL	400	21.97 ± 3.27	9.57 ± 0.40***	8.25 ± 3.17***	7.38 ± 4.21***	10.55 ± 1.41***
NTL	400	20.12 ± 2.37	8.52 ± 0.25***	7.55 ± 2.35***	6.02 ± 2.26***	5.22 ± 3.16***
DTL	400	21.77 ± 2.32	9.95 ± 0.26***	10.73 ± 2.23***	8.25 ± 2.40***	8.70 ± 2.35***
MTL	200	25.67 ± 3.41	12.07 ± 0.92***	16.20 ± 1.48**	12.40 ± 1.66***	13.85 ± 1.45**
BTL	200	24.40 ± 1.41	10.78 ± 0.47***	18.22 ± 1.47***	14.08 ± 2.34***	15.30 ± 2.45***
NTL	200	25.15 ± 2.27	10.73 ± 0.41***	15.63 ± 2.16***	13.55 ± 2.22***	11.45 ± 2.18***
DTL	200	22.20 ± 0.20	12.37 ± 0.25***	17.75 ± 1.24***	13.23 ± 1.29***	12.92 ± 2.25***
MTL	100	24.13 ± 2.30	18.37 ± 0.28**	21.13 ± 3.40**	17.37 ± 2.25*	18.18 ± 3.23**
BTL	100	23.52 ± 3.42	13.40 ± 1.42***	20.08 ± 2.26**	19.33 ± 3.37**	16.58 ± 3.40**
NTL	100	24.20 ± 2.45	12.43 ± 2.46***	18.52 ± 2.41***	16.18 ± 2.34*	14.55 ± 1.41***
DTL	100	25.45 ± 3.35	18.77 ± 2.40**	19.83 ± 0.29***	19.27 ± 1.17**	17.53 ± 3.43***

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (n = 6) were considered significant compared to the control sample in the One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

Table 3
Effect of *n*-hexane and other fractions (*T. leucostaphylum*) on Hole Cross Test (HCT) in mice.

Treatment	Dose (mg/kg, p.o.)	Number of hole cross				
		0 min	30 min	60 min	90 min	120 min
Control	10 mg/mL	22.83 ± 1.67$^{\#}$	18.33 ± 2.09$^{\#}$	16.50 ± 1.56$^{\#}$	19.33 ± 1.67$^{\#}$	20.67 ± 1.68$^{\#}$
Diazepam	2	22.83 ± 2.13	6.50 ± 0.62***	5.17 ± 0.70***	3.33 ± 0.42***	4.83 ± 0.79***
MTL	400	23.15 ± 0.50	11.40 ± 1.43***	9.55 ± 1.03***	7.32 ± 0.43***	6.45 ± 0.34***
BTL	400	21.97 ± 0.27	9.57 ± 0.40***	8.25 ± 0.17***	6.38 ± 0.21***	8.55 ± 0.41***
NTL	400	20.12 ± 0.37	8.52 ± 0.25***	7.55 ± 0.35***	5.02 ± 0.26***	4.22 ± 0.16***
DTL	400	21.77 ± 0.32	6.95 ± 0.26***	5.73 ± 0.23***	4.25 ± 0.40***	7.70 ± 0.35***
MTL	200	25.67 ± 0.41	12.07 ± 0.92***	10.20 ± 0.48**	8.40 ± 0.66***	11.85 ± 0.45***
BTL	200	24.40 ± 0.41	10.78 ± 0.47***	12.22 ± 0.47**	10.08 ± 0.34***	10.30 ± 0.45***
NTL	200	25.15 ± 0.29	9.73 ± 0.41***	13.63 ± 0.16**	9.55 ± 0.22***	13.45 ± 0.18**
DTL	200	22.20 ± 0.23	8.37 ± 0.25***	14.75 ± 0.24*	13.23 ± 0.29**	12.92 ± 0.25**
MTL	100	24.13 ± 0.38	12.37 ± 0.28**	11.13 ± 0.40**	12.37 ± 0.25**	15.18 ± 0.23*
BTL	100	19.52 ± 0.62	11.40 ± 0.42**	13.08 ± 0.26*	14.33 ± 0.37**	16.58 ± 0.40*
NTL	100	24.20 ± 0.73	13.43 ± 0.46*	12.52 ± 0.41*	15.18 ± 0.34*	14.55 ± 0.41*
DTL	100	25.45 ± 0.83	14.77 ± 0.40*	13.83 ± 0.29*	16.27 ± 0.17	13.53 ± 0.43**

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (n = 6) were considered significant compared to the control sample in the One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

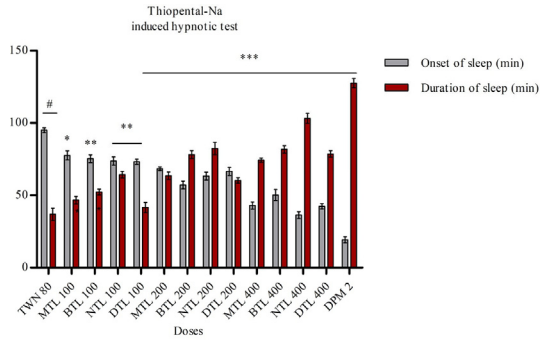

Fig. 3. Effect of *n*-hexane and other fractions (*T. leucostaphylum*) on thiopental sodium-induced hypnotic test in mice. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (n = 6) were considered significant compared to the control sample in the One Way Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

ranking of the binding affinity of the phytoconstituents of the n-hexane fraction and 5I6X receptor is as follows: 9,12,15-octadecatrienoic acid > 9-octadecenal > phytol > phytyl acetate > 6,10,14-trimethylpentadecan-2-one > 13-docosenoic acid > 13-tetradecynoic acid > methyl palmitate. 9,12,15-octadecatrienoic acid showed the highest binding affinity to the 5I6X receptor with a docking score of −5.4 kcal/mol. Again, phytol scored the highest binding affinity to the human serotonin transporter. Therefore, phytyl acetate attained peak binding affinity (-6.8 kcal/mol) when it reacted with the human cytochrome P450 CYP2C9 receptor. The order of the binding interaction at this receptor is: phytyl acetate > 13-docosenoic acid > 6,10,14-trime thylpentadecan-2-one > methyl linoleate > phytol > 13-tetradecy noic acid > 9,12,15-dctadecatrienoic acid > methyl palmitate > 9-octadecenal. The docking results have been illustrated in Table 6 and Fig. 5.

4. Discussion

The traditional healers of indigenous communities in Bangladesh use several plants/plant parts, with often unknown ingredients, for the treatment of different diseases/illnesses including cancer/tumors and psychological disorders, often with promising anecdotal results but which have never been screened. Hence, this study aimed to investigate the anxiolytic, antidepressant, and cytotoxic activities of a Bangladeshi ethnomedicinal plant, *T. leucostaphyllum* through in vivo, in vitro, and *in silico* approaches. We also tried to identify the compounds from the active extracts through GC–MS techniques.

In this study, an acute toxicity test postulated that the plant extract was safe at a therapeutic dose of up to 2000 mg/kg of body weight. In the force swimming test, mice were forced to swim in a confined area where there was no way to escape. It is widely recognized that the immobility seen in rodents while swimming represents the same kind of despairing behavior observed in human depression and that antidepressant medications effectively reduce immobility in mice (Kulkarni and Dhir, 2007).

Although the precise mechanism underlying FST's antidepressant-like activity in mice has yet to be identified, it is hypothesized that N-methyl-D-aspartate (NMDA) could be involved in the pharmacological effect of our extracts because Glutamate N-methyl-d-aspartate receptor (NMDAR) antagonists have been shown to affect the FST in mice. Depression and other neuropsychiatric illnesses have been linked to NMDARs, a subtype of glutamate receptors involved in synaptic plasticity and memory formation. Antidepressant-like effects have been seen in studies where NMDAR antagonists decrease the immobility duration in

Table 4
Calculated IC_{50} of the tested sample of *T. leucostaphylum*.

Samples	IC_{50} (µg/ml)				
	Cancer cell lines				
	MGC 803	A549	HeLa	U-251	MCF-7
NTL	77.03	47.35	269.6	**14.3**	478.5
DTL	244.2	136.8	437	169	92.17
BTL	Not detectable	Not detectable	427.9	528.6	308.4
MTL	276.1	377.5	681	786.5	179.3
Cisplastin	21.21	5.76	22	13.38	21.88

Table 5
List of chemical compounds identified by GC–MS in the *n*-hexen (NTL) fractions of *T. leucostaphylum*.

Sl. No.	Retention time (Min)	Initial time	Finishing time	Relative Peak area	Relative Peak area (%)	Relative Peak height	Relative Peak height (%)	Phytochemical name	Molecular formula	Molecular weight
01	32.208	32.153	32.253	250,743	2.96	88,773	4.32	Phytyl acetate	$C_{22}H_{42}O_2$	338
02	32.284	32.253	32.327	78,189	0.92	32,431	1.58	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268
03	33.086	33.047	33.133	63,186	0.74	24,252	1.18	Phytol	$C_{20}H_{40}O$	296
04	33.911	33.860	33.973	161,466	1.90	56,239	2.74	13-Docosenoic acid methyl ester	$C_{23}H_{44}O_2$	352
05	34.044	33.973	34.133	2,006,053	23.65	692,826	33.75	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270
06	34.829	34.760	34.927	856,874	10.10	249,696	12.16	Methyl Palmitic acid	$C_{16}H_{32}O_2$	256
07	38.846	38.747	38.947	1,520,773	17.93	343,782	16.75	9,12,15-Octadecatrienoic acid	$C_{19}H_{34}O_2$	294
08	39.048	38.947	39.240	2,481,252	29.25	379,993	18.51	Methyl elaidolinolenate	$C_{19}H_{32}O_2$	292
09	40.220	40.140	40.333	668,614	7.88	130,435	6.35	13-Tetradecynoic acid	$C_{15}H_{26}O_2$	238
10	40.506	40.393	40.620	396,048	4.67	54,488	2.65	9-Octadecenal	$C_{18}H_{34}O$	266

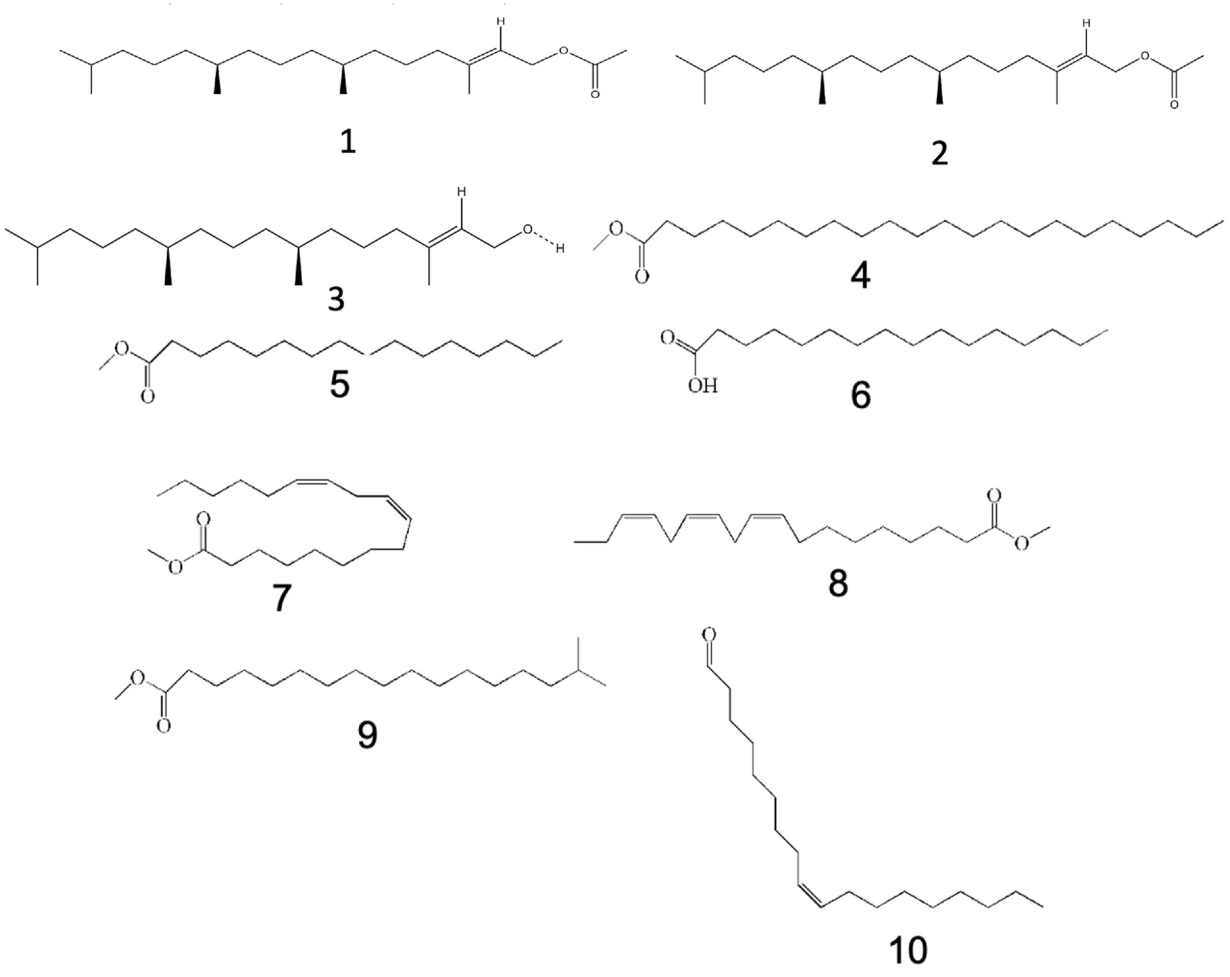

Fig. 4. Chemical structure of identified compounds from the *n*-hexane fraction of *T. leucostaphylum*.

the FST. For instance, ketamine, a non-competitive NMDAR antagonist, has been found to have fast and prolonged antidepressant effects in both animal models and human clinical studies (Ludka et al., 2013; Shahsavarian et al., 2014).

In addition, we tested the anxiolytic properties of the MTL, BTL, NTL, and DTL extracts in several anxiety models to see how efficient they were. A recent study found that the elevated plus-maze is one of the most commonly used animal anxiety models. Diazepam acts as a positive control and is believed to operate through the Gamma-aminobutyric acid $GABA_A$ receptor complex. Following previous research, diazepam enhanced the open-arm entrances and time spent in the open arms (Saavedra et al., 2006), confirming its anxiolytic effects. The effects of the aerial part extracts of MTL, BTL, NTL, and DTL were comparable with the anxiolytic effect of diazepam. Likewise, the extract also increases the frequency of head poking in the hole board test, which indicates an anxiolytic effect. The hole board test is a simple and frequently used technique for evaluating the reactions of animals'

Table 6
Docking score of the selected proteins and compounds for the anxiolytic, antidepressant, and cytotoxic activities.

Docking score						
Compounds	**PubChem ID**	**Anxiolytic**		**Antidepressant**		**Cytotoxic**
		4UUJ	**4K5Y**	**5I6X**	**6VRH**	**1OG5**
9,12,15-Octadecatrienoic acid	860	−5.4	−5.7	−5.4	−6.4	−5.6
9-Octadecenal	5,283,381	−4.6	−5.0	−5.3	−5.9	−5.2
13-Docosenoic acid	5,363,109	−5.3	−5.4	−4.8	−6.0	−6.2
13-Tetradecynoic acid	5,312,715	−4.6	−5.2	−4.8	−6.0	−5.7
Methyl palmitate	8181	−4.6	−4.9	−4.6	−5.8	−5.3
Methyl linoleate	5,284,421	−5.0	−5.6	−4.8	−6.1	−6.1
6,10,14-Trimethylpentadecan-2-one	10,408	−5.0	−5.6	−4.9	−6.4	−6.2
Phytol	5,280,435	−5.2	−5.5	−5.2	−6.7	−5.9
Phytyl acetate	637,195	−5.8	−5.9	−5.2	−6.1	−6.8
Diazepam/Paclitaxel	3016/36,314	−7.2	−5.7	−6.4	−6.3	−9.0

emotionality, anxiety, and responses to stress (Arenas et al., 2014). Head-dipping behavior (Chatterjee et al., 2011) is sensitive to changes in the animal's emotional state. It is an established protocol to examine an anxiolytic state in animals, reflecting the increase in head-dipping behavior. Animals are usually involved in head-dipping behavior if they are anxious. The findings show that MTL, BTL, NTL, and DTL extracts demonstrate antidepressant and anxiolytic effects at a dosage of 100–400 (mg/kg; b.w; p.o.).

Furthermore, the *n*-hexane and other fractions of *T. leucostaphyllum* (MTL, BTL, NTL, and DTL) have moderate sedative effects. In the thiopental sodium-induced test, the extracts of the plant were shown to enhance sleep induction in a dose-dependent manner, indicating that the crude samples had sleep-inducing properties. Thiopental sodium is a hypnotic drug and, with the proper dosage, increases GABA-mediated postsynaptic inhibition to increase hypnosis. Substances with CNS depressive action either reduce the onset time of sleep or increase the length of sleep (Alam et al., 2006). Additional studies confirmed that *n*-hexane and other fractions from the aerial parts of *T. leucostaphyllum* reduce locomotor activity in rodents. Because locomotor activity is a marker of CNS excitability, this reduction in spontaneous motor activity may be attributable to the plant extracts' sedative effect (Ali et al., 2015; Khan et al., 2014). All doses of the crude sample substantially reduced motility in mice. The impact of decreasing locomotor activity was seen during the second observation (30 min) and lasted until the fifth observation period (120 min). Moreover, the locomotion effects were dose-dependent and statistically significant ($p < 0.05$, 0.01, and 0.001).

Different anxiolytic, muscle relaxant, sedative-hypnotic, and sedative-hypnotic medicines are being studied to determine how they work by targeting $GABA_A$. Sedative and anxiolytic drugs, such as benzodiazepines, enhance GABA-mediated synaptic inhibition by increasing GABA action on the $GABA_A$ receptor (Biswas et al., 2021). GABA is a neurotransmitter that acts as an inhibitory neurotransmitter throughout the central nervous system. Our investigation clearly shows that the extract's potency is best at a higher concentration (especially at the doses of 200 and 400 mg/kg;b.w). It is almost equal to the impact of a well-known benzodiazepine, diazepam. Also, it is possible to infer that chemical components of the extract can alleviate the rats' anxiety through the same mode of action as benzodiazepines (Dolai et al., 2012). It may be affecting the mice through glutamatergic, noradrenergic, and serotoninergic receptors (Dawson and Tricklebank, 1995).

Furthermore, the extract's ability to potentiate pentobarbital-induced hypnosis provides additional evidence of its central depressant activity. This effect may be attributed to an action on the central mechanisms involved in regulating sleep or inhibition of the pentobarbital metabolism (Chindo et al., 2003; Amos et al., 2005). Similarly, the extract substantially reduced the exploratory behavior of mice, as shown by a decrease in the head-dip test results. The test is a measure of exploratory behavior that indicates the presence of sedative activity in various drugs (Amos et al., 2005).

As a result, it is conceivable that MTL, BTL, NTL, and DTL work by potentiating GABAergic inhibition in the CNS through membrane hyperpolarization, which results in a reduction in the firing rate of primary neurons in the brain, or that the extract acts by directly activating the GABA receptor (Kolawole and Makinde, 2007). Numerous studies have shown that plants containing flavonoids, saponins, and tannins are beneficial in treating a wide range of CNS diseases (Bhattacharya and Satyan, 1997). An earlier investigation into the phytoconstituents of plants suggested that many flavonoids and neuroactive steroids seemed to have a strong binding affinity for the $GABA_A$ receptors. It is possible that they can act similarly to benzodiazepines. Atypical antidepressants, as well as tricyclic antidepressants, serotonin-specific reuptake inhibitors, MAO inhibitors, and other atypical antidepressants, can be detected by the TST and FST tests (Agrawal et al., 2011). Cessation of their recurrent escape tendency occurred when they were put in an inescapable cylinder of water during FST, and also when their tails suspended the rodents during TST. In the assessment of prospective antidepressant drugs, it is believed that this reduction in length of immobility caused by MTL, BTL, NTL, and DTL has a high predictive value for the management of depression and anxiety (Dolai et al., 2012). Again, NTL (100, 200, and 400 mg/kg: b.w.) shows better results than other test samples, which may be mediated by interaction with the adrenergic and dopaminergic systems. The exact mechanisms of antidepressant action are yet to be investigated at this moment due to the fact that the chemical composition of the NTL is still unknown. The antidepressant, anxiolytic, and sedative-hypnotic activities of NTL may be attributed to the presence of phytoconstituents in the plant extracts.

We also tested the cytotoxicity of plant extracts from the chosen plant at seven different concentrations using the MTT (3-(4,5 -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on a panel of cancer cell lines. Preliminary screening results showed that the extent of inhibition varied between the types of cell lines and also between the types of extract used. The plant extracts, namely: *n*-hexane, DCM, n-butanol, and aqueous fractions, were tested for cytotoxic activities at different concentrations. Among all fractions, the *n*-hexane fraction was dominant in activity against U-251 cell lines (IC_{50}-14.3 μg/mL). To the best of our knowledge, this species' antitumor/anticancer activity is reported herein for the first time.

A quantitative phytochemical analysis of NTL also reported that it contains substantial amounts of phytyl acetate (2.96%), 2-pentadecanone, 6,10,14-trimethyl (0.92%), phytol (0.74%), 13-docosenoic acid, methyl ester (1.90%), hexadecanoic acid methyl

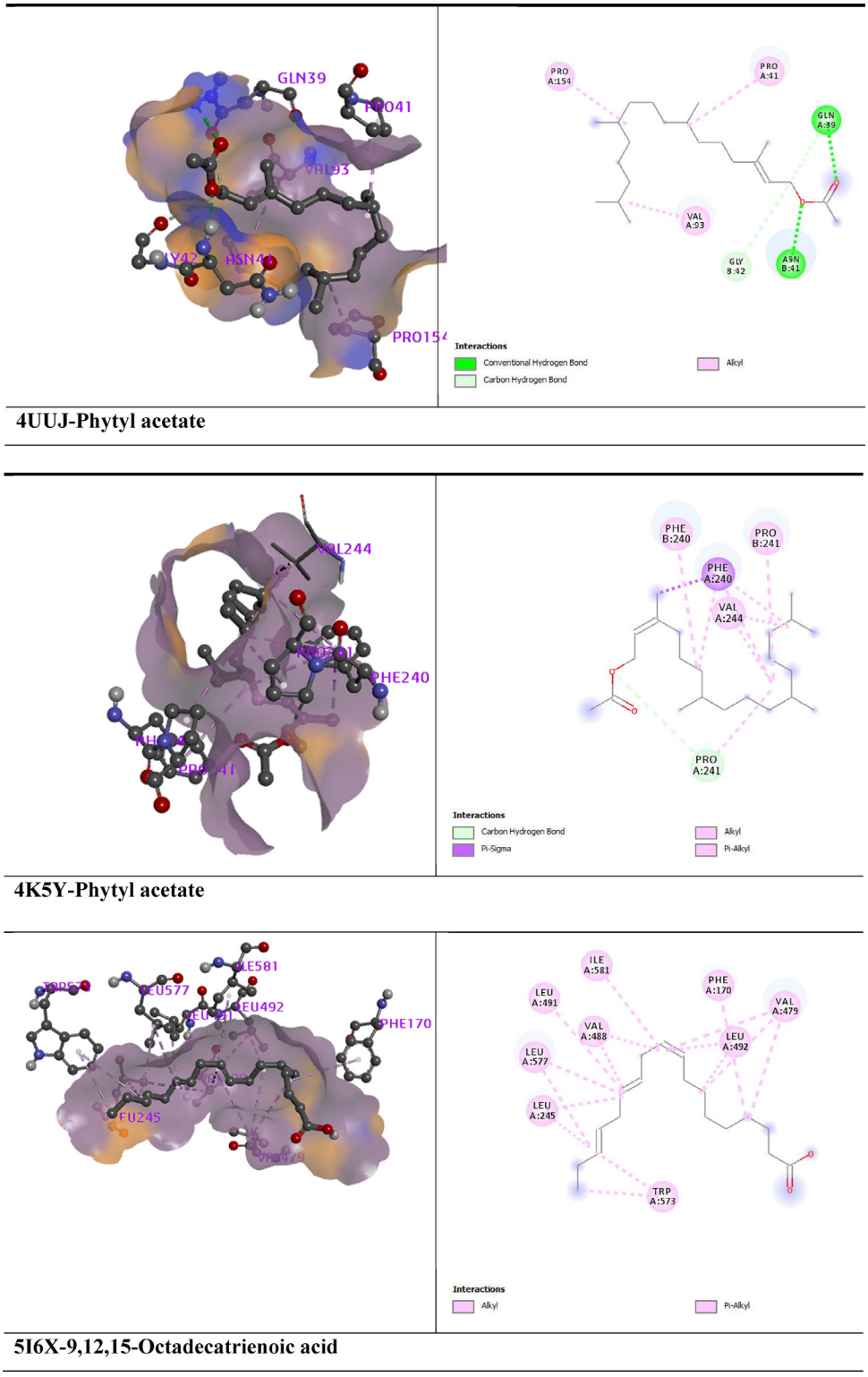

Fig. 5. Presentation of best binding mode (3D and 2D) of the selected proteins with the identified Phytoconstituents of the *n*-hexane fractions of *T. leucostaphyllum*.

ester (23.65%), methyl palmitic acid (10.10%), linoleic acid methyl ester (17.93%), methyl elaidolinolenate (29.25%), 13-tetradecynoic acid (7.88%) and 9,12,15-octadecatrienoic acid (4.67%).

Another major part of creating novel medications is the computer-aided drug design (CADD) approach. There are two kinds of drug design methods that are often used: structural drug design and ligand-based drug design (Śledź and Caflisch, 2018). In a prior study, researchers utilized ligand-based relationships to choose a lead compound with a high affinity for the potassium channel, human corticotropin-releasing factor, human serotonin transporter, and human cytochrome P450 CYP2C9 receptors. In this

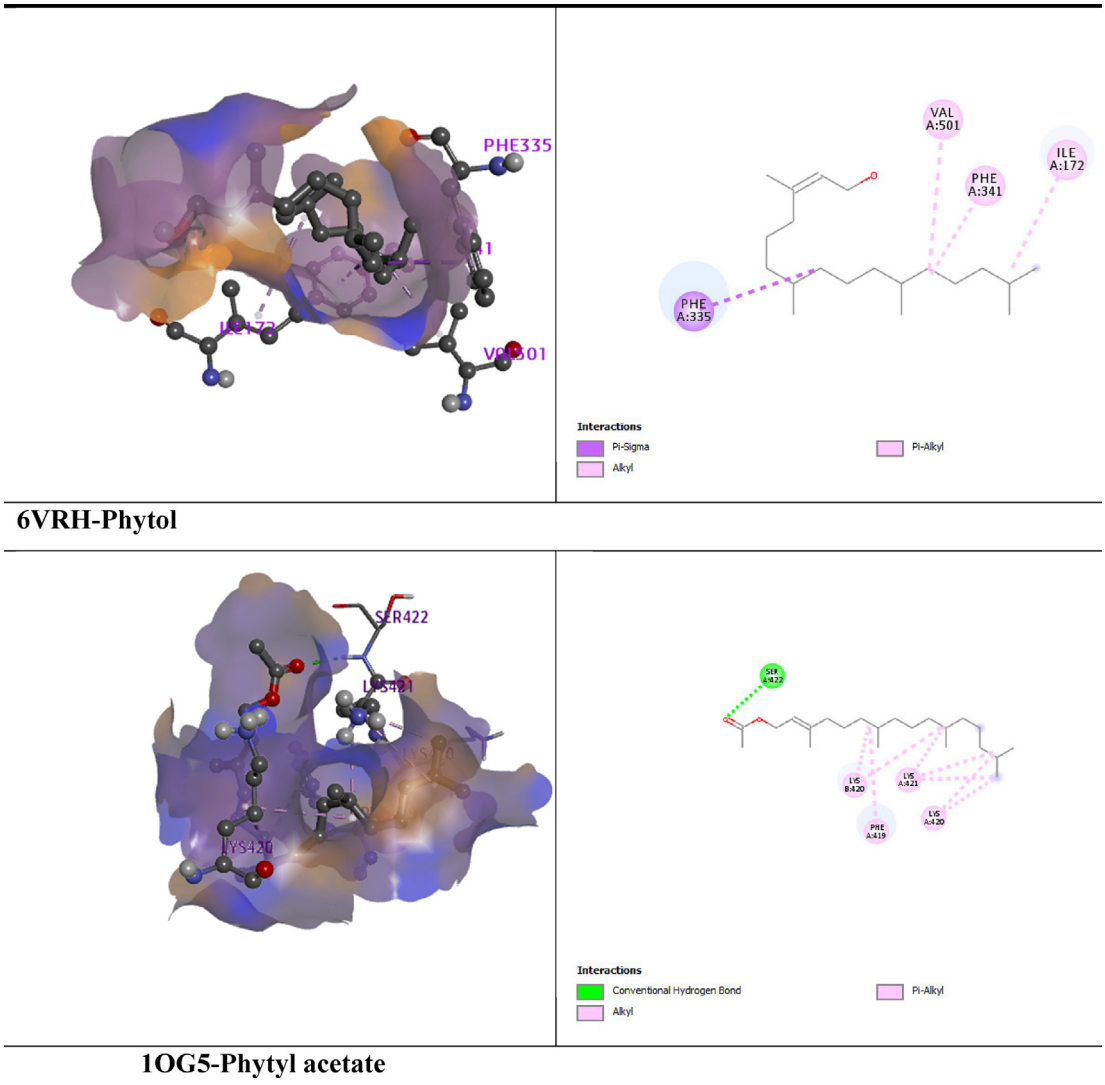

Fig. 5 (*continued*)

research, molecular docking was carried out for nine compounds of *T. leucostaphyllum,* and the results demonstrated that phytyl acetate, 9,12,15-octadecatrienoic acid, and phytol have a better probability of being claimed as drug candidates as they showed the best binding interactions to the receptors. However, it can be recommended that they have a better interface with the selected receptors than the other bioactive constituents. Therefore, more experiments to confirm the pharmacological implications of these isolated substances are required.

5. Conclusion

To sum up, it was distinguished that the test samples, especially *n*-hexane fractions, have promising neuropharmacological impacts on the investigated models, and it also portrays a hopeful finding on the cancer cell lines. Furthermore, the discovered chemical demonstrated decisive actions on antidepressant, anxiolytic, and cytotoxic receptors. The overall result provides a fundamental judgment of the neuropharmacological and anticancer capabilities of the samples. However, advanced research is recommended on these samples to elucidate whether they are exceptional therapeutic candidates against depression, anxiety, and cancer disease.

List of Compounds

Compound Name.	Molecular Formula.
Phytol acetate.	$C_{22}H_{42}O_2$.
2-Pentadecanone, 6,10,14-trimethyl.	$C_{18}H_{36}O$.
3,7,11,15-Tetramethyl-2-hexadecen-1-ol.	$C_{20}H_{40}O$.
13-Docosenoic acid methyl ester.	$C_{23}H_{44}O_2$.
Hexadecanoic acid methyl ester.	$C_{17}H_{34}O_2$.
n-Hexadecanoic acid.	$C_{16}H_{32}O_2$.
Linoleic acid methyl ester.	$C_{19}H_{34}O_2$.
9,12,15-Octadecatrienoic acid methyl ester.	$C_{19}H_{32}O_2$.
Heptadecanoic acid, 16-methyl-, methyl ester.	$C_{15}H_{26}O_2$.
cis-9-Octadecenal.	$C_{18}H_{34}O$.

Ethical consideration

All of the pharmacological tests were done in line with the ethical guidelines in the Declaration of Helsinki 2013. It was decided to euthanize the animals after they were maintained and handled in accordance with the standards established by the Swiss Academy of Medical Sciences and the Swiss Academy of Sciences and in accordance with the guidelines published in the Guidelines for the Euthanasia of Animals: 2013 Edition. Therefore, Jahangir Nagar University, Savar, Bangladesh [BBECJU/M2018(3)1] authorized the research procedure after reviewing the entire regulations.

6. Consent for publication

All the authors have given their unanimous consent to publishing their findings.

7. Availability of data and material

All the data are included in the manuscript.

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CRediT authorship contribution statement

Sajib Rudra: Conceptualization, Investigation, Methodology, Software, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Mohammad Omar Faruque:** Conceptualization, Investigation, Methodology, Formal analysis, Resources, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Afroza Tahamina:** Investigation, Data curation, Formal analysis. **Nazim Uddin Emon:** Investigation, Methodology, Software, Data curation, Formal analysis, Writing – original draft. **Ibrahim Khalil Al Haidar:** Data curation, Formal analysis, Writing – review & editing. **Shaikh Bokhtear Uddin:** Resources, Project administration, Validation.

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 material

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