Article

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Biological Assessment, GC-MS Analysis, and Molecular Docking Investigation on the Neuropharmacological, Anti-Diarrhoeal, and Cytotoxic Properties of *Ficus semicordata* Fruits

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Background: ***Ficus semicordata*** **Buch. is a well-known ethnomedicinal plant that is used to treat various ailments such as colic pain, urogenital difficulties, gastrointestinal disorders, visceral blockage, leprosy, jaundice, diabetes, and hepatitis. This study aims to investigate the phytochemical contents of the metabolites extracted (methanol) from the fruits of *Ficus* (*F.*) *semicordata*, and determine their neuropharmacological, anti-diarrhoeal, and cytotoxic potencies, using *in vivo*, *in vitro*, and *in silico* methods.**
Methods: The pharmacological properties of methanol extract of *Ficus semicordata* fruits (MEFSF) were assessed at different concentrations and its toxicity was determined using the *in vitro* brine shrimp lethality test. Tail suspension and forced swimming tests were used to investigate the antidepressant activity of MEFSF in mice and elevated plus maze and hole board test models were used to uncover its anxiolytic potentiality. The *in vivo* anti-diarrhoeal properties of MEFSF were tested on castor oil-induced diarrhoea and gastrointestinal motility models. Gas chromatography-mass spectrometry (GC-MS) analysis was conducted using a mass spectrometer. Based on the GC-MS analysis, 19 phytochemicals were investigated using molecular docking techniques against various target proteins to determine whether they mediate cytotoxic, depressive, anxiolytic, and anti-diarrhoeal effects.
Results: MEFSF exhibited moderate toxicity (median lethal dose (LD_{50}): 267.23 µg/mL). In the antidepressant assessment, MEFSF demonstrated a significant ($p < 0.0001$) dose-dependent decrease in immobility compared to fluoxetine. Similarly, MEFSF exhibited a dose-dependent reduction in anxiolytic-like behaviour in mice, with a 400 mg/kg dose exhibiting vigorous activity. MEFSF also significantly inhibited motility in both anti-diarrhoeal models, with a 400 mg/kg dose exhibiting highly significant ($p < 0.0001$) suppression. The GC-MS analysis revealed 81 bioactive components. Seven phytochemicals exhibited a strong affinity for various target proteins in the molecular investigation. Notably, beta-D-glucopyranose and 4-O-beta-D-galactopyranosyl manifested a high affinity for hER, K^+ channel, SERT3, and M3MAR and exhibited cytotoxic, anxiolytic, antidepressant, and anti-diarrhoeal potential.
Conclusion: The findings indicate that MEFSF can potentially contribute to the development of innovative anti-cancer, neuropharmacological, and anti-diarrhoeal treatments. However, additional research is necessary to explore this possibility.

Keywords: *Ficus semicordata*; antidepressant; anti-diarrhoeal; anxiolytic; cytotoxic; GC-MS analysis; molecular docking

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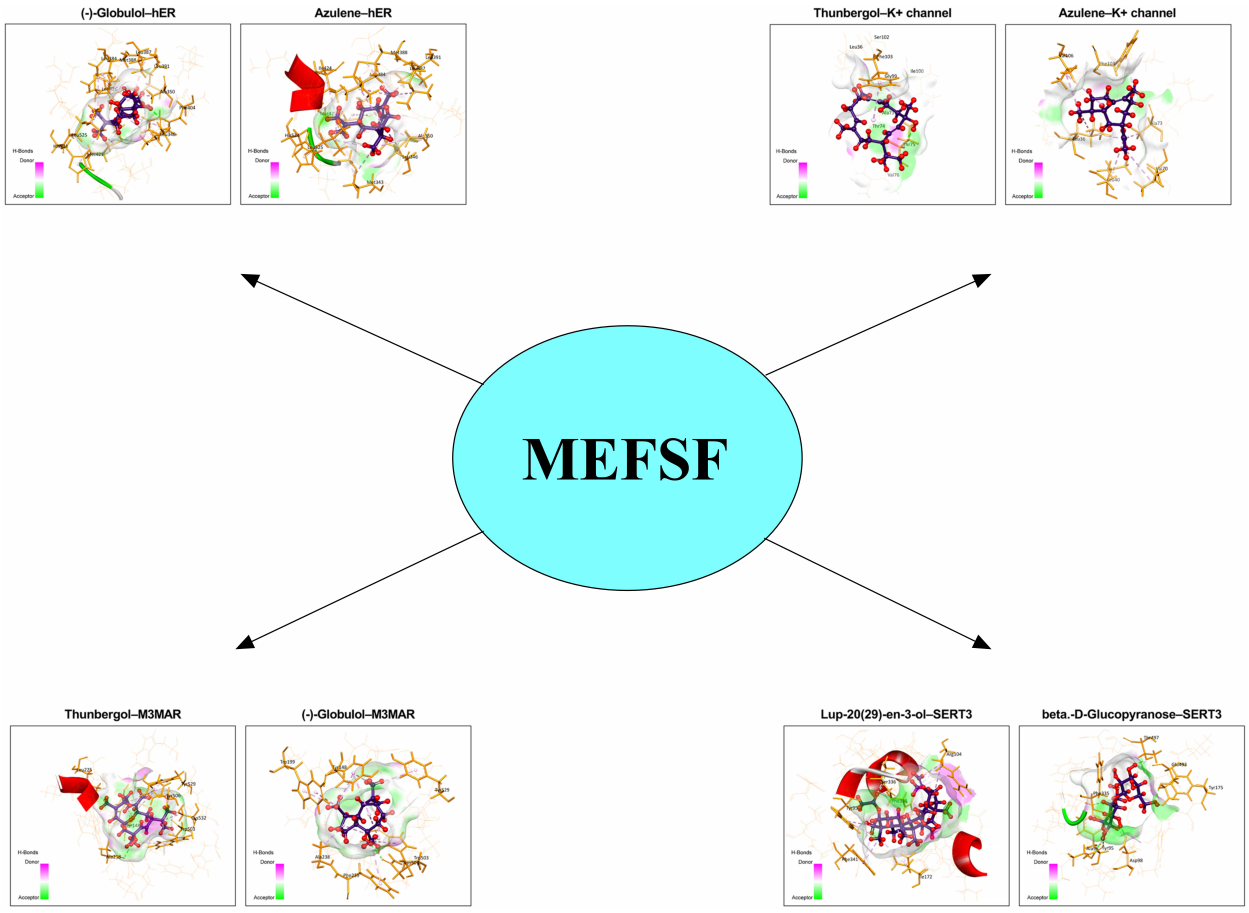

Graphical Abstract.

Introduction

Since primitive times, medicinal plants have been utilised as component resources in drug development and synthesis [1]. In both developed and developing countries, medicinal plants are increasingly being used to treat illnesses owing to the inconvenience and side effects of synthetic medicines [2]. Statistics reveal that most (80%) of the global population depends on herbal medications, and over 25% of modern medicines have plant origins [3,4]. Several bioactive compounds (e.g., glycosides, polyphenol, saponins, vitamin C, steroids) are extracted from plants with potential pharmacological and biological activity, including neuroprotective, antidepressant, antioxidant, anxiolytic, thrombolytic, anti-inflammatory, antimicrobial, hepatoprotective, and anti-cancer activity [5–7].

Major depressive disorder (MDD) is a commonly occurring and varied psychiatric disorder that is distinguished as an emotional and cognitive illness (e.g., energy loss, cognitive impairment, and apathy). It is a leading psychiatric disorder that affects more than 450 million individuals globally, according to the World Health Organization (WHO). MDD causes hypersomnia, anorexia, sleep deprivation, and physical and mental distraction, which can lead to suicidal tendencies, thereby increasing the number of mortalities each year [8–12]. Unfortunately, medication to cure depression is yet to be developed, but research has found that the outcome of molecular and cellular activities influences several genetic, psychological, biological, and environmental factors [13]. Researchers have hypothesised that depression may occur owing to the loss of monoamines (e.g., norepinephrine, serotonin, dopamine, etc.) in the brain. Several antidepressant drugs have been developed and employed to manage depression, but their success rate is low and they have significant drawbacks [14–16]. It is essential to develop new antidepressants that can reduce these drawbacks, and researchers and pharmacologists are increasingly turning to traditional medicines to treat various psychiatric disorders [17].

One of the most prevalent infectious disorders is diarrhoea, which is caused by an imbalance between the secretion and absorption of nutrients in the bowel, leading to increased face volume. Annually, approximately 1.8 million (3.2%) people die due to diarrhoea, of which 1.5 million are children. In developing countries, diarrhoeais a significant problem owing to poor sanitation and a lack of proper cleanliness, and bacteria such as *E. coli*, *S. typhi*, and *S. aureus* can cause significant harm [18–20]. According to the WHO, 17% of Bangladeshi children under the age of five suffer from diarrhoea. Owing to the widespread use of con-

ventional and herbal medicine in developing countries, the WHO encourages the use of traditional medicines to manage these infectious diseases [21,22].

Ficus semicordata Buch. is a tiny to average-sized tropical tree that belongs to the family Moraceae. It is used as medicine and food throughout South Asia and is locally known as drooping fig (English), dumur (Bangla), or khunia (Hindi) [23,24]. Traditionally, the fruits of this plant are used to treat several health issues, including colic pain, urogenital problems, abdominal troubles, visceral obstruction, leprosy, jaundice, diabetes, and hepatitis [25]. The stem bark is also used to manage numerous disorders (e.g., ulcers, pregnancy, wounds, dysentery, leprosy, liver complications, and gastric and bladder issues) [26]. The fruit and leaves of this plant provide several nutritional benefits and have antioxidant activity [27]. The fruit and roots of this plant have the most therapeutic potential, with 16 different functions, whereas the stem bark, leaves, and latex have 14, 9, and 7 functions, respectively. Additionally, 40 and 25 categories of internal and external activities, respectively, have been reported in a review [28]. However, despite its various beneficial applications, until now, no scientific investigations have been carried out to determine its potential for treating neuropsychiatric disorders and diarrhoeal diseases. In this study, we aim to show the pharmacological potential (antidepressant, anxiolytic, antidiarrhoeal, and cytotoxic activity) of *Ficus (F.) semicordata* (fruits) through qualitative and quantitative analyses.

Methods & Materials

Drugs & Chemicals

To conduct the experiment, potassium acetate, methanol, aluminum chloride, sulfuric acid, sodium carbonate, and hydrochloric acid were collected from (R99, 55, 38, 34, 47, 43) Sigma Aldrich Co., St. Louis, MO USA. Castor oil (110, WELL'S health care, Spain) and Vincristine sulfate (L221, Beacon Pharmaceuticals Ltd., Dhaka, Bangladesh) were purchased for this investigation. Diclofenac sodium, diazepam, loperamide hydrochloride, and fluoxetine hydrochloride has chosen to collect from Square Pharmaceutical Limited. (L201, K22, F29, F33, Dhaka, Bangladesh). In this study, several analytical reagents with established references were employed.

Collection, Identification & Preparation of Crude Extract

In February 2019, from Nakapa at Ramgarh Upzilla in Khagrachari (Chittagong Hill Tracts Area), fruits of *F. semicordata* were collected and identified by a well-known taxonomist. Fresh fruits of the *F. semicrodata* were cut into little pieces and left to air dry in the shade for approximately 15 days. Once dried, the fruit pieces were ground into a coarse powder and preserved in an airtight bottle. The coarse powder was then soaked in methanol for a week at room temperature, with regular shaking and stirring. After this period, the mixture was filtered using Whitman filter paper. After filtration, the solvent (methanol) was meticulously removed through evaporation. This evaporation process was conducted at a controlled temperature of 40ºC, enabling the transformation of the solvent into vapor and leaving behind a slurry or viscous mass containing the concentrated constituents extracted from the dried fruits. Finally, the isolated extract was preserved for further pharmacological testing.

GC-MS Analysis

Using a mass spectrometer (TQ 8040, Shimadzu Corporation, Japan) and a gas chromatograph (GC-17A, Shimadzu Corporation, Kyoto, Japan) with a capillary column (Rxi-5 ms; 0.25 film, 30 m long, internal diameter 0.32 mm) coated with DB-1 (J&W) and a specific volume of methanol extract of *Ficus semicordata* fruits (MEFSF) analysis by gas chromatography-mass spectrometry (GC-MS). The oven was initially configured to operate at 70 °C for 0 minutes. Subsequently, the temperature was raised to 150 °C at a rate of 10 °C per minute for 5 minutes, 200 °C at a rate of 12 °C per minute for 5 minutes, and 220 °C at a rate of 12 °C per minute with a hold period of 10 minutes. The detector temperature was set at 280 °C, while the injector temperature was maintained at 230 °C; the electron impact mode was established at a 70 eV ionization voltage for the method of electron ionization; and the column had a 0.6 mL/min helium gas flow rate at constant pressure (90 kPa). Then, a sample volume of 1 microliter was injected in injection mode and ran for 50 minutes. The compounds were identified in the peak areas of the GC-MS dataset by comparison with NIST & WILEY libraries [29,30].

In Vitro Pharmacological Study

Qualitative Phytochemical Screening

With the standard procedures, the phytochemical analysis of MEFSF was conducted qualitatively [31]. Carbohydrates, cardiac glycosides, steroids, alkaloids, reducing sugar, polyphenols, triterpenoids, phenolic and flavonoid compounds were investigated from crude MEFSF extract.

In Vitro Cytotoxicity Assay

The Brine Shrimp lethality bioassay test is the first and broadest technique for the bioactive composites of natural and synthetic derivatives. This study of MEFSF was performed by following the method of Meyer *et al.* [32]. Thirty-eight grams of sea salt (without iodine) were weighed, diluted in one liter of distilled water, and filtered to provide a clear solution for hatching shrimp eggs. After that, the brine shrimp eggs were placed in this artificial seawater and kept at room temperature with a constant oxygen supply. The brine shrimp eggs need two days to mature, known as nauplii. The crude extract was then organized for serial dilution, and several concentrations (10 to

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1000 µg/mL) were prepared after dissolving in DMSO (5 mg/mL). Several concentrations (0.125 to 10 µg/mL) of the standard drug (vincristine sulfate) were prepared as the preceding method and used as a positive control in this study. Then, ten live nauplii were taken in per experimental vial and incubated at room temperature for a day. The living nauplii were counted through a magnifying glass and noted. The percentage of mortality of nauplii was denoted according to the equation:

$$\text{Percentage (\%) of mortality } = \frac{N_0 - N_1}{N_0} \times 100$$

Where, N_0 = The number of nauplii taken; N_1 = The number of nauplii alive.

In Vivo Pharmacological Assay

Experimental Animals

Swiss Albino mice (total mice 48, weight: 28–32 grams; different sexes) were procured from Jahangir Nagar University, Savar, Dhaka, Bangladesh. These mice were kept in plastic cages (120 × 30 × 30 cm) with well-standard conditions (55–60% relative humidity, 25 ± 2 °C temperature) with proper food, water, and ventilation. They fulfilled a natural day-light cycle in the room. All the tests were conducted separately in a quiet room. The P&D committee (Pharm-P&D17/08'-19), Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh, sanctioned & authenticated the whole study protocol. According to the reference number IIUC/PHARM-AEC-150/20-2019, every experimental protocol that is concerned with this research was approved by the IAEC (Institutional Animal Ethical Committee), Department of Pharmacy, International Islamic University Chittagong, Chittagong 4318, Bangladesh [33]. All experimental mice were given 50–200 mg/kg of sodium pentobarbital for anesthesia of these studies and no mice died. In this report, all the sections were conducted through the guidelines of "Animal Research: Reporting of *In Vivo* Experiments" and the "Principles of the Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) and "National Animal Care Laws" were strictly imitated in time of animals handling.

Acute Oral Toxicity Test

"OECD Guidelines" were followed to conduct acute toxicity tests of this experiment [34]. Six Swiss albino mice were orally administered varying doses of the crude extract, ranging from 500 to 2000 mg/kg. Afterward, the mice were closely observed for 3–4 days, checking for allergic reactions like swelling, skin rashes, and itching and noting any instances of mortality.

Experimental Design

The experiment involved the random allocation of mice (both male and female, n = 6) into four distinct groups: a control group, a standard group, and two test groups. The crude extract of MEFSF was administrated to varying dosages (200 and 400 mg/kg, b.w., p.o., respectively), and 1% Tween in water was considered vehicles, which was administrated to the control group orally. The standard diazepam (1 mg/kg, b.w.; i.p.) was administrated to a joint group of mice for the experiments of elevated plus maze (EPM) test and hole board test (HBT) while fluoxetine (20 mg/kg, intraperitoneally) was utilized in the tail suspension test (TST) and forced swimming test (FST). The standard received the reference drug loperamide (5 mg/kg, intraperitoneally) in the anti-diarrheal test. The MEFSF doses were administered half an hour before the experiments, whereas the reference drugs (diazepam, fluoxetine, loperamide) were administered 15 minutes before the experiments.

Anti-Depressant Activity

Tail Suspension Test (TST). The tail suspension test was performed by Steru *et al*. [35] during the experiment with slight modification. Each group of mice was hung in a box (25 × 25 × 30 cm) for six minutes with the help of a sticky tape put about 1 centimeter from the tip of the tail. Earlier, 60 min of the experiment dosage was given, as described. The total immobile time was recorded during the final 5 min of the 7 min period induced by tail suspension. Mice were regarded as stationary when they demonstrated a complete lack of body movement, hanging in a passive and motionless state.

Force Swimming Test (FST). Swiss albino mice of either sex were separately forced to swim in a jar (25 × 15 × 25 cm) filled with 15 cm of water at 25 ± 1 °C temperature with the intention of forced swimming test (FST) evaluation [36]. Two divided sessions were implemented for this experiment, in which the former session was conducted a day before the second session to allow the mice to become accustomed to the environment. All the mice were given doses earlier than 60 min of the study described. All mice required swimming continuously for 7 min, and their immobility was observed and recorded throughout the final 5-min interval of the test. Calmness in each mouse was identified when it ceased struggling and remained afloat in the water, making only the necessary movements to keep its head above the surface. A decrease in immobility time demonstrates an antidepressant effect.

Anxiolytic Activity

Elevated Plus Maze (EPM) Test. Pellow *et al*. [37] the method was considered to perform the test. The equipment comprises two open arms (5 × 10 cm) and two closed arms (5 × 10 × 15 cm) placed on a floor (5 × 5 cm) to give the

apparatus a plus sign appearance, which was positioned 40 cm above the floor, and the maze ground and walls were assembled from wood. Mice were administered in distinct doses mentioned, and every rodent was positioned facing one of the enclosed arms in the middle of the maze. The total number of mice that entered an open arm during the five minutes of the test was recorded. A mouse is considered to have entered an arm when it places all four paws on it.

Hole Board Test (HBT). The HBT experiment was performed by the described method [38]. For this test, a 40 cm × 40 cm × 25 cm in diameter board with 16 evenly spaced holes set up 25 cm above the floor. The dosing treatments for each animal group, and thirty minutes after receiving the test dose, mice were placed in the center of the board and allowed to move freely. Finally, each mouse was permitted to travel on the board and counted the number of head dips into the holes for 5 min.

Anti-Diarrheal Activity

Castor Oil Induced Anti-Diarrheal Test. Nwodo and Alumanah [39] method was used for this experiment with minor modifications [39]. The allocated mice fasted for 24 hours before receiving the dosage regimens detailed are mentioned for each group of mice (n = 6). Each animal received 0.5 mL of castor oil after an hour of treatment and then was placed into a separate cage with adsorbent paper (blotting paper) at the bottom. The diarrheal excretions (dry faces and wet faces) were recorded during the 4-h observation period for each mouse, and new blotting paper was substituted for the old one at the beginning of each hour. Finally, the percentage of inhibition (%) of defecation & diarrhea is calculated via the following equation:

$$\text{Inhibition of defecation } (\%) = \frac{A - B}{A} \times 100$$

A = mean number of defecations faces of the control group; B = mean number of defecations caused by extracts.

Gastrointestinal (GI) Motility Test by Charcoal Marker. The gastrointestinal (GI) motility test was approved utilizing the technique outlined by Mascolo *et al*. [40] with slight modification. All experimental mice were fasted for 24 hours before administration, and the treatment doses were mentioned. After one hour of administering treatment doses, the experimental mice got 1mL of charcoal solution (10% charcoal, 5% gum acacia) orally. Then, all experimental mice were given a high dose of sodium pentobarbital anesthesia after an hour. The distance moved by charcoal solution from pylorus to caecum was measured, and the percentage of inhibition and peristalsis index was calculated according to this equation:

$$\%\ \text{of Inhibition} = \frac{\text{Distance Travel by the control (cm)} - \text{Distance travel by the test groups (cm)}}{\text{Distance travel by the control (cm)}} \times 100$$

Statistical Analysis

The data was expressed as the mean ± SEM (n = 6). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests in GraphPad Prism version 7.0 (GraphPad Software, Boston, MA, USA); statistical significance was attributed to experimental results with a *p*-value less than 0.05. Linear regression equation method was applied to detect lethal concentration 50 (LC_{50}) of plant extract and reference standard drug.

In Silico Molecular Docking Studies

The phytochemicals found in MEFSF from GC-MS analysis were tested against four different receptors using a molecular docking technique to gain possible molecular interactions and binding modes at the target proteins' active sites. For this study, 19 critical compounds identified by GC-MS analysis of MEFSF were chosen as an extensive literature review revealed that these compounds had seldom been explored for their pharmacological actions. This study was executed on Schrödinger suite-Maestro (version 11.8, Schrödinger, LLC, NY, USA, 2018).

Ligand Preparation

The two-dimensional (2D) chemical structures of the MEFSF compounds were retrieved in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The 2D structures were imported into the Schrödinger Maestro, and by employing Schrödinger's LigPrep tool, the structures were neutralized at pH (7.0 ± 2.0) via Epik (version 4.6) [41] and subsequently reshaped into three-dimensional (3D) structures under OPLS3e force field.

Protein Preparation

3D crystallographic structures of the voltage-gated potassium (K^+) channel, human estrogen receptor (hER), M3 muscarinic acetylcholine receptor (M3MAR) and ts3 human serotonin transporter (SERT3) were acquired in protein data bank (PDB) format from the RCSB Protein Data Bank (http://www.rcsb.org) with PDB accession code 3ERT [42], 4UUJ [43], 5I6X [44], and 4U14 [45]. The protein structures were imported into the Schrödinger Maestro and subjected to preprocess using Protein Preparation Wizard: bond orders were assigned, hydrogens (H_2) were added, zero-order bonds to metals and disulfide bonds were created, missing side chains and loops were filled by Prime (version 5.4), het states were generated at pH (7.0 ± 2.0) using Epik, water molecules and cofactors were eliminated. The proteins were further refined by optimization of the orientation of the H_2-bonded groups. Finally, minimization was accomplished to set a maximal heavy atom RMSD (root mean square deviation) to 0.30 Å.

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Grid Generation and Molecular Docking

Glide (version 8.1), embedded in Schrödinger Maestro, was employed to design a receptor grid and to conduct docking studies [46,47]. Flexible docking studies were conducted under default parameters using Glide's Standard Precision (SP) score function, and the highest negative docking score ranked ligands possessing maximal favorable binding energetics against the respective target proteins. The co-crystallized ligands were re-docked with individual proteins to validate the docking protocol, and the RMSD values were examined. Molecular interactions between the receptor-ligand complex were visualized and analyzed via BIOVIA Discovery Studio Visualizer v20 (BIOVIA, San Diego, CA, USA).

Prediction of Pharmacokinetic and Toxicological (ADME/T) Profiles

Drug features, i.e., physicochemical properties postulated in Lipinski and Veber rules, of the potential bioactive compounds of MEFSF were computed using the SwissADME web tool (http://www.swissadme.ch) [48]. Lipinski's rule of five claims that effective absorption or permeation is more feasible when a drug candidate possesses molecular weight (MW) $\leq$500 Da, number of H2 bond acceptors (nHBA) $\leq$10, number of H2 bond donors (nHBD) $\leq$5, and lipophilicity (LogP) $\leq$5 [49]. Veber's rules suggested two more relevant descriptors: the number of rotatable bonds (nRB) $\leq$10 and topological polar surface area (TPSA) $\leq$140 Å2 [50]. Besides drug-likeness descriptors, pharmacokinetic parameters (i.e., absorption, distribution, metabolism, excretion and toxicity (ADME/T)) profiles of the compounds have been predicted from the pkCSM ADME/T descriptor algorithm protocol (http://biosig.unimelb.edu.au/pkcsm/prediction) [51]. The absorption properties of the compounds were estimated according to Caco2 permeability, intestinal absorption, and P-Glycoprotein (P-gp) substrate. To anticipate the distribution profile of the compounds, factors such as the steady-state distribution volume (VDss), blood-brain barrier (BBB) permeability (logBB), and central nervous system (CNS) permeability (logPS) were assessed. The metabolism profile was investigated based on inhibiting cytochromes P450 (CYP) enzymes, such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Excretion was evaluated regarding the total clearance model and the renal OCT2 substrate. Toxic effects were assessed based on Ames toxicity, hepatoxicity, and oral rat acute toxicity.

Results

GC-MS Analysis

GC-MS is usually applied for direct analysis of components. In this experiment, 81 compounds were traced in the GC-MS analysis, as shown in Table 1 and Fig. 1. Most chemical entities were fatty acids, organic compounds, esters, alcohols & phenols. In contrast, the most enormous chemical agents with their retention time were thunbergol (33.802), azulene 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) (32.800), (-)-globulol or agarospirol (32.800), alpha-linolenic acid, trimethylsilyl ester (32.593), urs-12-en-3-ol, acetate, (3.beta.) (31.969), betulin (31.969), lup-20(29)-en-3-ol, acetate, (3.beta.) (31.969), lupeol (31.969). Those entities isolated from MEFSF could be responsive for neuropsychiatric and cancer management. Nineteen major compounds were selected from this analysis to investigate molecular docking.

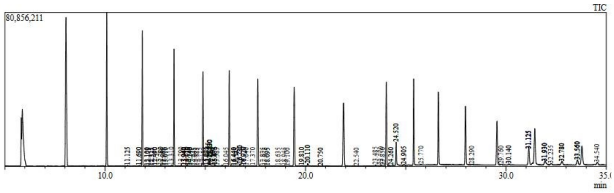

Fig. 1. Total ionic chromatogram (TIC) of the methanol extract of *Ficus semicordata* fruits of using gas chromatography-mass spectrometry (GC-MS).

In Vitro Pharmacological Study

Qualitative Phytochemical Screening

To ensure the existence of secondary plant metabolites, we conducted a qualitative phytochemical study of MEFSF. Positive results were obtained for carbohydrates, reducing sugars, alkaloids, polyphenols, terpenoids, cardiac glycosides, and flavonoids, as shown in Table 2.

In Vitro Brine Shrimp Lethality Bioassay

Different concentrations of MEFSF (31.25–1000 µg/mL) were tested for cytotoxic activity based on the dosage, considering its cytotoxic effect against brine shrimp. The results are shown in Fig. 2. The LC_{50} value was 267.23 µg/mL (Regression equation: Y = 0.0821X + 28.06). In contrast, the standard drug vincristine sulphate has an LC_{50} value of 2.15 µg/mL (Regression equation: Y = 7.6315X + 33.536).

In Vivo Pharmacological Study

Acute Toxicity Test

The acute toxicity profiles of MEFSF were investigated using Swiss albino mice. The crude extract was orally administered to the mice in a single dose of 500–2000 mg/kg, which did not result in any toxicity or fatalities. The mice did not exhibit any behavioural toxicity 72 h posttreatment. Thus, according to the findings of this screening, MEFSF is safe for practical use, and dosage levels of 200 mg/kg and 400 mg/kg (b.w.,p.o) were used in the subsequent *in vivo* investigations.

Table 1. Chemical compounds identified from the fruits of methanol extract of *Ficus (F.) semicordata* using gas chromatography-mass spectrometry (GC-MS) analysis.

Sl. No	Compound Name	Chemical Formula	MW	Retention time	m/z	Area	Conc.(ug/mL)	Nature
01	Propane, 2-methoxy-2-methyl-	$(CH_3)_3COCH_3$	88.15	11.124	73.00	250477	0.057	Alkane
02	3-Pentanol, 2,4-dimethyl-	$C_7H_{16}O$	116.20	11.859	73.00	17504152	3.959	Secondary Alcohol
03	Nonadecane	$C_{19}H_{40}$	268.5	12.109	57.00	74665	0.017	Alkane
04	Octadecanoic acid, 2-oxo-, methyl ester	$C_{19}H_{36}O_3$	312.4873	12.109	57.00	74665	0.017	Fatty Acid
05	2-Nonen-1-ol	$C_9H_{18}O$	142.24	12.109	57.00	74665	0.017	Aliphatic alcohol
06	Glucitol, 6-O-nonyl-	$C_{15}H_{32}O_6$	308.41	11.859	73.00	16336812	3.695	Nonylphenol
07	Trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170.29	11.859	73.00	16336812	3.695	Phenol
08	Trans-2-Nonen-1-ol	$C_9H_{18}O$	142.24	11.859	73.00	16336812	3.695	Aliphatic alcohol
09	Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethyl ester	$C_{16}H_{30}O_4Si_3$	370.66	12.486	73.00	142171	0.032	Ester
10	Androsta-3,5-dien-3-ol, 17-acetyl-3-O-(t-butyldimethylsilyl)-	$C_{27}H_{44}O_2Si$	444.72	12.486	73.00	142171	0.032	Steroids
11	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334.4498	12.783	149.00	258318	0.058	Fatty Acid
12	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	12.783	149.00	258318	0.058	Phthalic acid
13	4-Tetradecanol	$C_{14}H_{30}O$	214.39	13.330	73.00	161408	0.037	Myristyl alcohol
14	3-Octanol, 3,7-dimethyl-	$C_{10}H_{22}O$	158.28	13.330	73.00	161408	0.037	Phenol
15	4-Dodecanol	$C_{12}H_{26}O$	186.33	13.443	73.00	14680643	3.320	Fatty alcohol
16	1H-Cycloprop(e)azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-	$C_{15}H_{24}$	204.35	13.443	57.00	230874	0.052	Alkene
17	Oleic Acid	$C_{18}H_{34}O_2$	282.5	14.074	73.00	266619	0.060	Fatty acid
18	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.40	14.074	73.00	266619	0.060	Saturated fatty acid
19	n-Decanoic acid	$C_{10}H_{20}O_2$	172.26	14.074	73.00	266619	0.060	Fatty Acid
20	Undecanoic acid	$C_{11}H_{22}O_2$	186.29	14.074	73.00	266619	0.060	Carboxylic acid
21	Epinephrine, (.beta.)-, 3TMS derivative	$C_{18}H_{37}NO_3Si_3$	399.7	14.074	73.00	266619	0.060	Methyl-silyloxy-ethan-amine
22	Octanal, 7-methoxy-3,7-dimethyl-	$C_{11}H_{22}O_2$	186.29	14.074	73.00	266619	0.060	Aldehyde
23	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	14.074	73.00	266619	0.060	Fatty Acid
24	Digitoxin	$C_{41}H_{64}O_{13}$	764.9	14.074	73.00	266619	0.060	Cardiac glycoside
25	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.074	73.00	266619	0.060	Saturated fatty acid
26	Galactitol	$C_6H_{14}O_6$	182.17	14.886	73.00	12178794	2.754	Sugar alcohol
27	Malic acid, 3TBDMS derivative	$C_{22}H_{48}O_5Si_3$	476.87	14.886	73.00	12178794	2.754	Fatty acid
28	Butanedioic acid, 2-[(tert-butyldimethylsilyl)oxy]-, bis	$C_{22}H_{48}O_5Si_3$	476.87	14.886	73.00	12178794	2.754	Fatty acid
29	1-Docosene	$C_{22}H_{44}$	308.6	15.233	55.00	441273	0.100	Alkene
30	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5	15.171	67.00	577834	0.131	Fatty acid
31	9,11-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.5	15.171	67.00	577834	0.131	Fatty acid
32	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.5	15.232	79.00	561716	0.127	Fatty acid
33	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298.9	15.232	79.00	561716	0.127	Fatty acid chloride
34	11,14,17-Eicosatrienoic acid, methyl ester	$C_{20}H_{34}O_2$	306.5	15.232	79.00	561716	0.127	Fatty acid
35	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.5	15.232	79.00	561716	0.127	Fatty acid
36	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	$C_{18}H_{32}O$	264.4	15.232	79.00	561716	0.127	Phenol

Table 1. Continued.

Sl. No	Compound Name	Chemical Formula	MW	Retention time	m/z	Area	Conc.(ug/mL)	Nature
37	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.4	15.233	55.00	441273	0.100	Ester
38	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.42	15.233	74.00	163832	0.037	Ester
39	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424.74	15.233	74.00	163832	0.037	Ester
40	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$	466.82	15.233	74.00	163832	0.037	Ester
41	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340.6	15.233	74.00	163832	0.037	Ester
42	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354.61	15.233	74.00	163832	0.037	Ester
43	8-Methyl-6-nonenoic acid	$C_{10}H_{18}O_2$	170.25	16.196	73.00	12082929	2.733	Fatty acid
44	E-11-Tetradecenol, trimethylsilyl ether	$C_{17}H_{36}OSi$	284.6	16.196	73.00	12082929	2.733	Ether
45	Undecanal	$C_{11}H_{22}O$	170.29	16.196	73.00	12082929	2.733	Undecyl aldehyde
46	Azacyclotridecan-2-one	$C_{12}H_{23}NO$	197.32	16.730	55.00	206847	0.047	Macrocyclic lactams
47	Formic acid, decyl ester	$C_{11}H_{22}O_2$	186.29	16.730	55.00	206847	0.047	Ester
48	Pentafluoropropionic acid, decyl ester	$C_{13}H_{21}F_5O_2$	304.29	16.730	55.00	206847	0.047	Ester
49	1-Heptafluorobutyryloxydecane	$C_{14}H_{21}F_7O_2$	354.30	16.730	55.00	206847	0.047	Ester
50	Cyclononanone	$C_9H_{16}O$	140.22	16.730	55.00	206847	0.047	Cyclic ketone
51	Decyl trifluoroacetate	$C_{12}H_{21}F_3O_2$	254.29	16.730	55.00	206847	0.047	Fluoropolymer
52	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281.5	17.620	73.00	13397469	3.030	Acid amide
53	Phloroglucitol	$C_6H_{12}O_3$	132.16	18.954	73.00	271036	0.061	Phenol
54	beta-D-Glucopyranose, 4-O-beta-D-galactopyranosy	$C_{12}H_{22}O_{11}$	342.30	19.436	73.00	13863230	3.135	Glycosides
55	1-Eicosanol	$C_{20}H_{42}O$	298.5	20.114	57.00	283171	0.064	Arachidyl alcohol
56	1-Tetradecanol	$C_{14}H_{30}O$	214.39	20.114	57.00	283171	0.064	Myristyl alcohol
57	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.55	20.114	57.00	283171	0.064	Ester
58	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50	20.114	57.00	283171	0.064	Ester
59	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.5	20.114	57.00	283171	0.064	Mono acyl-glycerols
60	Sorbitol	$C_6H_{14}O_6$	182.17	20.435	73.00	54822	0.012	Sugar alcohol
61	D-Galactonic acid, .gamma.-lactone	$C_6H_{10}O_6$	178.14	20.435	73.00	54822	0.012	Gluconolactone
62	(Z)-9-Tricosene	$C_{23}H_{46}$	322.6	23.495	57.00	128899	0.029	Muscamone
63	Tetradecanoic acid, 2,3-dihydroxypropyl ester	$C_{17}H_{34}O_4$	302.44	24.032	73.00	10979684	2.483	Ester
64	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337.6	24.522	59.00	3857386	0.872	Acid amide
65	Squalene	$C_{30}H_{50}$	410.7	24.522	69.00	1111067	0.251	Triterpene
66	Hexadecanoic acid, 2-bromo-	$C_{16}H_{31}BrO_2$	335.32	25.399	73.00	8873015	2.007	Bromo fatty acid
67	Methyl cholate	$C_{25}H_{42}O_5$	422.6	30.145	73.00	75085	0.017	Methyl ester
68	Dodecanedioic acid, bis(trimethylsilyl) ester	$C_{18}H_{38}O_4Si_2$	374.66	30.145	73.00	75085	0.017	Acid ester
69	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.7	31.136	43.00	1111837	0.251	Phytosterols
70	Cholesterol	$C_{27}H_{46}O$	386.7	31.136	43.00	1111837	0.251	Fat
71	Cholest-5-ene, 3-methoxy-, (3.beta.)-	$C_{28}H_{48}O$	400.68	31.136	43.00	1111837	0.251	Methyl ether

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Table 1. Continued.

Sl. No	Compound Name	Chemical Formula	MW	Retention time	m/z	Area	Conc.(ug/mL)	Nature
72	.beta.-Sitosterol	$C_{29}H_{50}O$	414.7	31.136	43.00	1111837	0.251	Phytosterols
73	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	$C_{28}H_{45}ClO_2$	449.1	31.136	43.00	1111837	0.251	Sterol
74	Lupeol	$C_{30}H_{50}O$	426.7	31.969	207.00	136485	0.031	Pentacyclic triterpenoid
75	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.8	31.969	207.00	136485	0.031	Triterpenoids
76	Betulin	$C_{30}H_{50}O_2$	442.7	31.969	207.00	136485	0.031	Triterpene
77	Urs-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.8	31.969	207.00	136485	0.031	Phenolic compound
78	.alpha.-Linolenic acid, trimethylsilyl ester	$C_{21}H_{38}O_2Si$	350.61	32.593	73.00	59114	0.013	Ester
79	(-)-GlobulolorAgarospirol	$C_{15}H_{26}O$	222.37	32.800	207.00	184808	0.042	Polyphenol
80	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)	$C_{15}H_{24}$	204.35	32.800	207.00	184808	0.042	Mancude carbo-bicyclic
81	Thunbergol	$C_{20}H_{34}O$	290.5	33.802	207.00	602108	0.136	Polyphenol

MW, molecular weight.

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Table 2. Result of phytochemical screening of methanol extract of *Ficus semicordata* (MEFSF).

Phytochemicals	Test types	Appearance	Results
Carbohydrates	Molisch's test	Reddish color ring form	++
Reducing sugar	Benedict's test	Reddish color precipitate form	++
	Fehling's test	Red precipitate form	++
Alkaloids	Wagner test	Reddish-brown color form	+
	Mayer's test	Pale-yellow color form	++
Polyphenols	Ferric cyanide test	Blue-green color form	++
Triterpenoids	Salkowski's test	Redish violet color form	+
Cardial Glycosides	Legal test	Brown color form	+
Flavonoids	Lead acetate test	Fluorescence-yellow color form	++

++: Rapidly present; +: Present.

Table 3. The effect of anti-diarrheal activity of MEFSF on castor oil induced diarrhea in mice (feces count).

	Castor oil induced diarrhea test			
Treatment	Total number of faces	Inhibition (%)	Total number of diarrheal faces	Inhibition (%)
Control	13.4 ± 0.46	-	9.93 ± 0.87	-
Loperamide	5.2 ± 0.33^a	61.19%	2.08 ± 0.22^a	79.05%
MEFSF 200	8.33 ± 0.88^b	37.84%	3.91 ± 0.74^b	60.62%
MEFSF 400	6.83 ± 0.54^b	49.03%	2.75 ± 0.14^a	72.31%

The results are expressed as the mean ± SEM (n = 6), whereas $^b p < 0.001$ and $^a p < 0.0001$ are considered statistically significant. Using a one-way ANOVA analysis of variance (Dunnett's test), the statistical analysis compared the results to the negative control (1% Tween-80) in GraphPad Prism version 7.0. MEFSF, methanol extract of *Ficus semicordata* fruits.

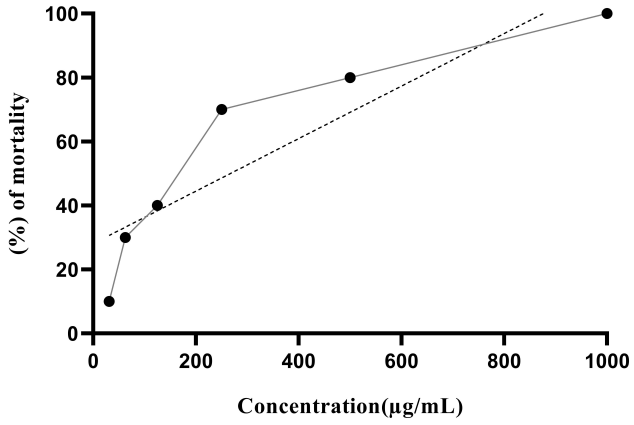

Fig. 2. Percentage of brine shrimp mortality at different concentrations of methanol extract of *Ficus semicordata* fruits (MEFSF).

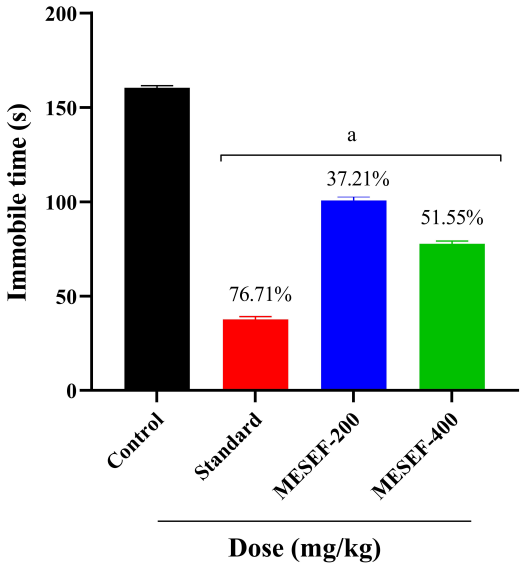

Fig. 3. Antidepressant activity of MEFSF on tail suspension tests in mice. The results are expressed as the mean ± SEM (n = 6), where $^a p < 0.001$ is considered statistically significant. Using a one-way ANOVA analysis of variance (Dunnett's test), the statistical analysis compared the results to the negative control (1% Tween-80) in GraphPad Prism version 7.0.

Antidepressant Activity

Tail Suspension Test. Fig. 3 presents the results of the immobility time in mice dosed with MEFSF. As shown, compared to the control group, there was a significant ($p < 0.0001$) dose-dependent decrease in immobile times of 100.66 ± 1.96 s (51.55%) and 77.66 ± 1.51 s (37.21%) for dosage levels of 400 mg/kg and 200 mg/kg, respectively. Similarly, mice administrated with fluoxetine (20 mg/kg) exhibited a significant ($p < 0.0001$) decrease in the immobility time of approximately 76.71% (37.67 ± 1.44 s).

Forced Swimming Test. The forced swimming test was used to study the antidepressant effect of the crude extract. Both dosage levels (200 mg/kg and 400 mg/kg, p.o.) significantly ($p < 0.0001$) decreased the immobility times, which

were 78.67 ± 4.09 s (33.71%) and 60.33 ± 3.52 s (49.16%) respectively, compared to that of the control (118.67 ± 2.20 s). Likewise, mice treated with 20 mg/kg of fluoxetine exhibited a significant ($p < 0.0001$) decrease in immobility time to 29.67 ± 2.90 s (74.99%), as shown in Fig. 4.

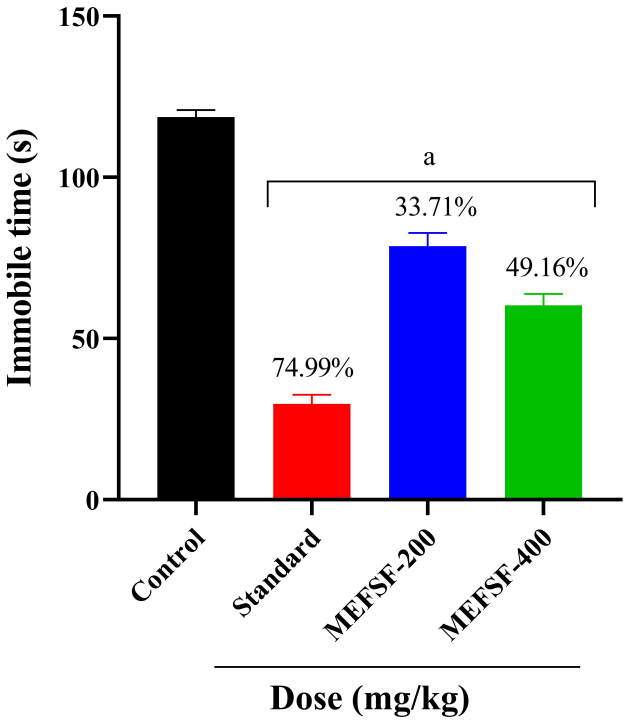

Fig. 4. Antidepressant activity of MEFSF in a forced swimming test (FST) in mice. The results are expressed as the mean ± SEM (n = 6), where $^{a}p < 0.001$ is considered statistically significant. The statistical analysis was followed by one-way analysis of variance (Dunnett's test) compared to the negative control (1% Tween-80) using GraphPad Prism version 7.0.

Anxiolytic Activity

Elevated Plus Maze (EPM) Test. The results of the EPM test revealed that mice treated with two MEFSF dosages – 400 mg/kg and 200 mg/kg – exhibited significant ($p < 0.05$) dose-dependent increases in their percentage of entry into the open arm, with values of 75.06% ± 2.79% and 62.94% ± 1.78%, respectively, compared to the control group. Notably, MEFSF increased ($p < 0.001$) the percentage of time spent in the open arm; for dosage levels of 200 mg/kg and 400 mg/kg, the mice spent 71.60% ± 3.29% and 78.39% ± 1.80% of the time, respectively, in the open arm. Diazepam (dosage of 1 mg/kg) also exhibited a noteworthy ($p < 0.0001$) increase in the percentage of entry and time spent in the open arm, with values of 77.02% ± 2.07% and 87.69% ± 1.61%, respectively. The results are shown in Fig. 5.

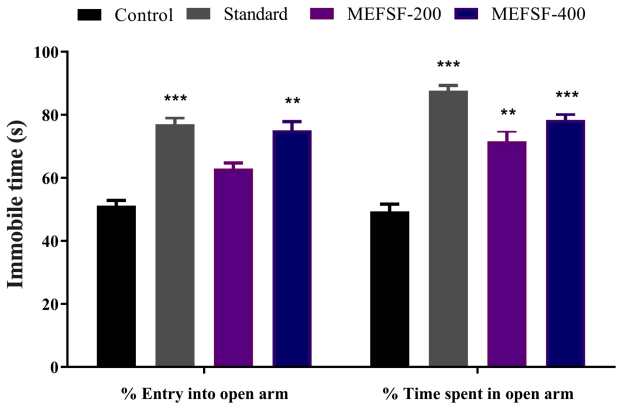

Fig. 5. The anxiolytic behavior of MEFSF and diazepam (standard) in mice's elevated plus maze (EPM) test. The results are expressed as the mean ± SEM (n = 6), where $^{**}p < 0.01$ and $^{***}p < 0.001$ are considered statistically significant. Using a one-way ANOVA analysis of variance (Dunnett's test), the statistical analysis compared the results to the negative control (1% Tween-80) in GraphPad Prism version 7.0.

Hole Board Test (HBT). The results of the HBT for MEFSF are shown in Fig. 6. The results indicated that 200 mg/kg and 400 mg/kg doses of MEFSF increased the head dipping of the mice compared to the control group, with values of 28.33 ± 2.02 and 38.67 ± 1.76 ($p < 0.05$), respectively. Correspondingly, 1 mg/kg of standard diazepam resulted in a significant ($p < 0.001$) increase in head dipping (58.66 ± 1.20).

Anti-Diarrhoeal Activity

Castor oil Induced Anti-diarrhoeal Test. Diarrhoea was apparent in all the control group mice for 4 h after applying castor oil. MEFSF exhibited a noteworthy ($p < 0.001$) dose-dependent reduction in diarrhoea over 4 h, as shown in Table 3. The 400 mg/kg dose had a more significant inhibition of 72.31%, which is comparable to the inhibition of the reference medication, loperamide (79.05%). At a dosage level of 200 mg/kg, MEFSF had an inhibition of 60.62%. Compared to the negative control group, the experimental findings indicated that the crude extract provides considerable ($p < 0.001$) anti-diarrhoeal activity against castor oil-induced diarrhoea.

Gastrointestinal (GI) Motility Test Using Charcoal Marker. Table 4 indicates that all the extract doses employed in this test resulted in a significant ($p < 0.01$) dose-dependent inhibition of the charcoal plugin in the mice. The reference medication loperamide had a similar inhibiting effect (51.91%). Furthermore, the 400 mg/kg dose of the crude extract resulted in higher inhibition (39.88%) than the 200 mg/kg dose (18.65%).

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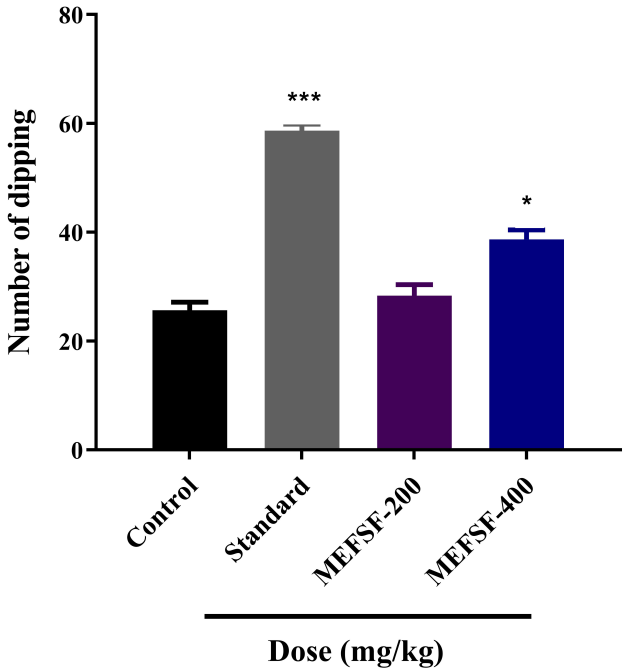

Fig. 6. The anxiolytic potentiality of MEFSF in a hole board test (HBT) in mice. The results are expressed as the mean ± SEM (n = 6), where $^{*}p < 0.05$ and $^{***}p < 0.001$ are considered statistically significant. The statistical analysis was followed by a one-way analysis of variance (Dunnett's test), and the results were compared to the negative control (1% Tween-80) using GraphPad Prism version 7.0.

Table 4. The effect of *F. semicordata* with reference to Loperamide on intestinal motility in mice by using charcoal as a marker.

Castor oil induced diarrhea test				
Treatment	Total length of intestine (cm)	Distance travel by charcoal (cm)	Peristalsis Index (%)	Inhibition (%)
Control	55.6 ± 0.85	47.12 ± 0.87	84.74 ± 1.88	-
Loperamide	52.66 ± 0.33^{d}	22.66 ± 1.45^{a}	43.04 ± 2.79^{a}	51.91%
MEFSF 200	60.01 ± 0.73^{c}	38.33 ± 1.76^{d}	63.89 ± 2.19^{b}	18.65%
MEFSF 400	64.52 ± 0.57^{a}	28.33 ± 1.2^{a}	44.27 ± 1.63^{a}	39.88%

The results are expressed as the mean ± SEM (n = 6), where $^{d}p < 0.05$, $^{c}p < 0.01$, $^{b}p < 0.001$ and $^{a}p < 0.0001$ are considered statistically significant. Using a one-way ANOVA analysis of variance (Dunnett's test), the statistical analysis compared the results to the negative control (1% Tween-80) in GraphPad Prism version 7.0. MEFSF, Methanol extract of *Ficus semicordata* fruits.

In-Silico Molecular Docking

This study used bioinformatics modeling to expect the molecular mechanisms that may observe the pharmacological effects of MEFSF and explore potential therapeutic agents. The findings of the molecular docking have been listed in Table 5. 19 phytocompounds observed via GC-MS analysis from MEFSF were first docked against the hER (PDB ID: 3ERT), a receptor implicated in cancer pathogenesis. Among the enlisted compounds, (-)-Globulol and Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) were assessed to have the highest binding strength at the active site of the hER with docking scores of –8.54 kcal/mol and –8.141 kcal/mol, respectively, which were significantly higher than the docking score of the reference standard anticancer drug vincristine sulfate (docking score: –4.036 kcal/mol). Besides that, Tetradecanoic acid, 2,3-dihydroxypropyl ester (docking score: –6.651 kcal/mol), Thunbergol (docking score: –7.386 kcal/mol), beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl (docking score: –5.678 kcal/mol), Glucitol, 6-0-nonyl- (docking score: –5.473 kcal/mol), Trans-2-Nonen-1-ol (docking score: –4.606 kcal/mol), Lup-20(29)-en-3-ol, acetate, (3.beta.)- (docking score: –4.991 kcal/mol), Hexadecanoic acid, 2-bromo- (docking score: –4.429 kcal/mol), Urs-12-en-3-ol, acetate, (3.beta.)- (docking score: –4.715 kcal/mol), 13-Docosenamide (Z)- (docking score: –4.546 kcal/mol), and 3-Pentanol, 2,4-dimethyl (docking score: –4.39 kcal/mol) also had stronger affinity to bind against the hER when compared to the docking score of vincristine sulfate.

Then, selected compounds were again docked against the K^{+} channel (PDB ID: 4UUJ) to investigate the possible phytochemicals and mechanisms underlying MEFSF's anti-anxiety effect. Among the studied compounds, Thun-

Table 5. The docking scores of the selected phytochemicals from MEFSF against hER (3ERT), K^+ channel (4UUJ), SERT3 (PDB ID: 5I6X), and M3MAR (4U14) for cytotoxic activity, anxiolytic activity, antidepressant activity, and antidiarrheal activity, respectively.

Compound	PubChem CID	Protein Docking score (kcal/mol)			
		hER	K^+ channel	SERT3	M3MAR
3-Pentanol, 2,4-dimethyl-	11752	−4.39	−4.451	−4.597	−5.896
Glucitol, 6-O-nonyl-	552730	−5.473	−3.766	−4.906	−6.542
Trans-2-Undecen-1-ol	5365004	−2.809	1.37	−0.446	−0.025
Trans-2-Nonen-1-ol	5364941	−4.606	−2.455	−2.933	−2.964
4-Dodecanol	66291	0.153	0.038	−0.497	−1.242
Malic acid, 3TBDMS derivative	528581	−3.607	−1.154	−4.43	−6.842
8-Methyl-6-nonenoic acid	5365959	−3.695	−2.913	−2.812	−4.021
E-11-Tetradecenol, trimethylsilyl ether	5366871	−1.308	0.058	−1.336	−2.663
Undecanal	8186	0.679	1.675	0.855	0.229
9-Octadecenamide, (Z)-	5283387	−1.592	−0.391	−2.392	−3.98
beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl	69301022	−5.678	−4.504	−7.71	−7.666
Tetradecanoic acid, 2,3-dihydroxypropyl ester	79050	−6.651	−3.311	−4.236	−5.768
13-Docosenamide, (Z)-	5365371	−4.546	−4.268	−0.855	−7.806
Hexadecanoic acid, 2-bromo-	82145	−4.429	−3.147	−4.674	−6.364
Lup-20(29)-en-3-ol, acetate, (3.beta.)-	6432150	−4.991	−3.692	−8.12	−6.208
Urs-12-en-3-ol, acetate, (3.beta.)-	91746713	−4.715	−3.936	−6.52	−6.46
(-)-Globulol	12304985	−8.54	-	−6.831	−8.082
Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)	6432384	−8.141	−4.877	−6.684	−7.338
Thunbergol	5363523	−7.386	−5.353	−6.609	−8.35
Reference standard (Vincristine sulfate/Fluoxetine/Diazepam/Loperamide)	249332/3386/3016/3955	−4.036	−5.165	−9.426	−8.543

K^+, voltage-gated potassium; PDB, protein data bank.

bergol (docking score: –5.353 kcal/mol) demonstrated superior binding strength to the target protein compared to the standard anti-anxiety agent diazepam (docking score: –5.165 kcal/mol). In addition, Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyl phenyl) and beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl also showed significant affinity as a binder at the active site of the K^+ channel, with docking scores of respectively –4.877 kcal/mol and –4.504 kcal/mol.

The docking study for the antidepressant potential of the selected MEFSF compounds was conducted by targeting an essential transport protein, SERT3 (PDB ID: 5I6X), which has been linked to the pathogenesis of clinical depression. Lup-20(29)-en-3-ol, acetate, (3.beta.)-, with a docking score of –8.12 kcal/mol, possessed the highest binding affinity against SERT3 among the enlisted compounds, followed by beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl (docking score: –7.71 kcal/mol) and (-)-Globulol (docking score: –6.831 kcal/mol). The reference standard antidepressant fluoxetine exhibited a slightly higher docking score (–9.426 kcal/mol) against the target protein in comparison to the compound Lup-20(29)-en-3-ol, acetate, (3.beta.)-.

The M3MAR was finally used as a target (PDB ID: 4U14) in the docking study for antidiarrheal activity. A closer look at the docking scores showed that Thunbergol and Globulol possessed the best binding strengths at the active site of the M3MAR, with docking scores of –8.35 kcal/mol and –8.082 kcal/mol respectively, which is significant when compared to the docking score of the standard antidiarrheal agent, loperamide (docking score: –8.543 kcal/mol). Docking scores of 13-Docosenamide, (Z)-, beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl, and Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) against M3MAR were also found to be favorable when compared to loperamide. They achieved docking scores of –7.806 kcal/mol, –7.666 kcal/mol, and –7.338 kcal/mol, respectively. The vital binding interactions of the best-docked phytocompounds with the active site residues of the respective target proteins have been depicted in Figs. 7,8.

ADME/T Profiles

Tables 6,7 represent *in silico* ADME/T profiles of investigated compounds that demonstrated great potentiality in the molecular study. The bioavailability proper-

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Table 6. Physicochemical properties of the selected compounds from MEFSF for favorable oral bioavailability.

Physicochemical Properties	Compound(s)						
	B-DG	13-DSM	LUP	URS	(-)-Globulol	AZU	Thunbergol
Lipinski Rules							
MW[1]	342.30	337.58	468.75	468.75	222.37	204.35	290.48
nHBA[2]	11	1	2	2	1	0	1
nHBD[3]	8	1	0	0	1	0	1
LogP[4]	−3.39	6.77	7.67	7.43	3.42	4.31	4.75
Lipinski's violations[5]	2	1	1	1	0	0	0
Veber Rules							
nRB[6]	4	19	3	2	0	1	1
TPSA[7]	189.53	43.09	26.30	26.30	20.23	0	20.23

[1]MW, molecular weight (acceptable range: ≤500); [2]nHBA, number of H_2 bond acceptors (acceptable range: ≤10); [3]nHBD, number of H_2 bond donors (acceptable range: ≤5); [4]LogP, lipophilicity (acceptable range: ≤5); [5]Lipinski's violations, number of violations of Lipinski's rule of five (acceptable range: ≤1); [6]nRB, number of rotatable bonds (acceptable range: ≤10); [7]TPSA, topological polar surface area (acceptable range: ≤140 Å^2). B-DG, beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl; 13-DSM, 13-Docosenamide, (Z)-; LUP, Lup-20(29)-en-3-ol, acetate, (3.beta.)-; URS, Urs-12-en-3-ol, acetate, (3.beta.)-; AZU, azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl).

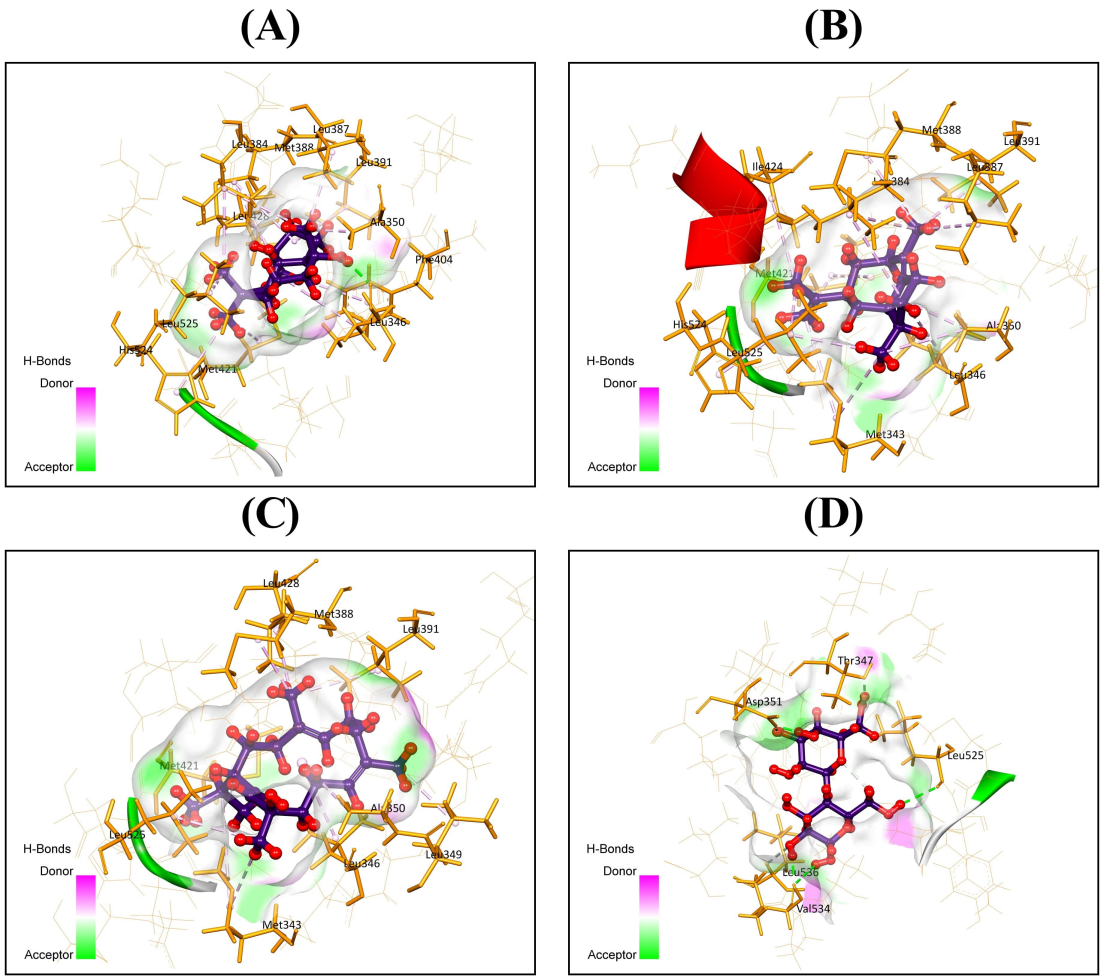

Fig. 7. Best ranked docking pose and interactions of (-)-Globulol (A), Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) (B), Thunbergol (C), and beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl (D) with active site residues of hER (PDB ID: 3ERT).

Table 7. Pharmacokinetics and toxicological (ADME/T) profiles of the potential bioactive compounds of MEFSF.

Parameter(s)	Compound(s)						
	B-DG	13-DSM	LUP	URS	(-)-Globulol	AZU	Thunbergol
Absorption							
Caco2 permeability[1]	−0.12	1.104	1.221	1.222	1.483	1.401	1.543
Intestinal absorption[2]	6.412	88.843	97.894	96.174	92.814	93.429	91.703
P-gp substrate	Yes	No	No	No	No	No	No
Distribution							
VDss[3]	0.203	0.173	−0.12	0.148	0.556	0.679	0.459
BBB permeability[4]	−1.024	−0.558	0.644	0.599	0.632	0.78	0.51
CNS permeability[5]	−4.683	−1.432	−1.754	−1.963	−2.176	−1.855	−2.614
Metabolism							
CYP1A2 inhibitor	No	Yes	No	No	Yes	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	Yes
CYP2C9 inhibitor	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No
Excretion							
Total clearance[6]	1.545	2.09	0.06	0.025	0.817	1.216	1.374
Renal OCT2 substrate	No	No	No	No	No	No	No
Toxicity							
Ames toxicity	No	No	No	No	No	No	No
Hepatotoxicity	No	No	No	No	No	No	No
Oral rat acute toxicity[7]	1.643	1.873	2.512	2.25	1.615	1.542	1.675

[1]Caco2 permeability (represented as log Papp in 10-6 cm/s, log Papp >0.90 = high permeability); [2]Intestinal absorption (represented in %, absorption rate <30% = poor); [3]VDss, steady-state volume of distribution (represented as log VDss in L/kg, log VDss <–0.15 = poor, log VDss >0.45 = high); [4]BBB permeability (represented as logBB, logBB >0.3 = highly permeable, logBB <–1 = poorly permeable); [5]CNS permeability (represented as logPS, logPS >–2 = penetrable, logPS <–3 = impenetrable); [6]Total clearance (predicted total clearance log(CLtot) is expressed in log(mL/min/kg)); [7]Oral rat acute toxicity (represented as median lethal dose (LD_{50}) in mol/kg). B-DG, beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl; 13-DSM, 13-Docosenamide, (Z)-; LUP, Lup-20(29)-en-3-ol, acetate, (3.beta.)-; URS, Urs-12-en-3-ol, acetate, (3.beta.)-; AZU, Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl). ADME/T, absorption, distribution, metabolism, excretion and toxicity; BBB, blood-brain barrier; P-gp, P-Glycoprotein; VDss, steady-state distribution volume; CNS, central nervous system; CYP, cytochromes P450; logBB, blood-brain barrier (BBB) permeability; logPS, central nervous system (CNS) permeability.

ties of the targeted compounds are characterized based on the physicochemical importance set out in LROF and Veber's rules. The findings show that Lipinski's violations of the analyzed compounds were between 0 and 2, whereas six of the seven compounds did not contravene more than one of LROF (Table 6). In addition to Lipinski's rules, five compounds under investigation, namely, Lup-20(29)-en-3-ol, acetate, (3.beta.)-, Urs-12-en-3-ol, acetate, (3.beta.)-, (-)-Globulol, Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyl phenyl), and Thunbergol complied with Veber's rules. In contrast, other compounds violated one of the two rules of Veber. The ADME/T studies also revealed that, aside from not being a P-gp substrate, all of the compounds, except beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl, are projected to have high Caco-2 I the body of rates. Besides, the estimated VDss volume, BBB permeability, and CNS permeability values of the compounds ranging from –0.12 to 0.679, –1.024 to 0.644, and –4.683 to –1.432, respectively, showed that the majority of the compounds are anticipated to possess favorable distribution, BBB permeability, and CNS penetration profiles. The prediction of metabolism revealed that none of the compounds inhibited more than one of the five CYP enzymes from the studied. Regarding excretion, the total clearance value of the compounds ranged from 0.025 to 2.09. Besides, all of the compounds originated to be non-substrate of renal OCT2. Finally, the toxicological profiles of the selected potential compounds were studied based on the following parameters: ames toxicity, hepatoxicity, and oral acute toxicity. The findings of the toxicological analysis revealed that all substances possess adverse Ames toxicity and hepatoxicity profiles. Besides, most compounds displayed lower acute toxicity in mice with a median lethal dose (LD_{50}) value ranging from 1.542 to 2.512 mol/kg.

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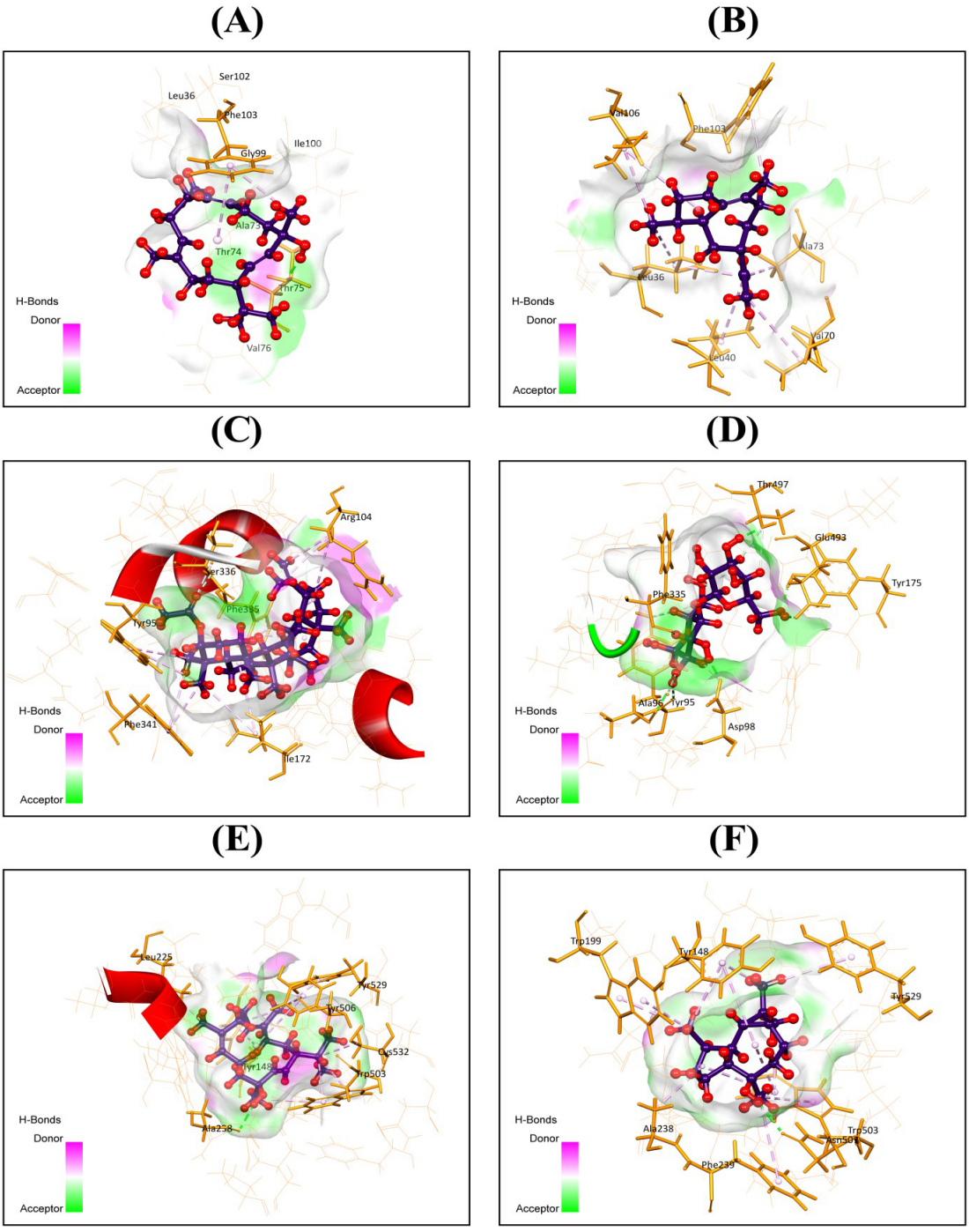

Fig. 8. Best ranked docking pose and interactions of Thunbergol (A) and Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl) (B) with active site residues of K^+channel, Lup-20(29)-en-3-ol, acetate, (3.beta.)- (C) and beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl (D) with active site residues of SERT3 (PDB ID:5I6X), and Thunbergol (E) and (-)-Globulol (F) with active site residues of M3MAR (PDB ID: 4U14).

Discussion

Medicinal plants play a crucial role as bioresources in various domains of contemporary medicine, including therapeutics, nutraceuticals, ayurvedic medicine, and traditional medicine [52,53]. The phytochemical screening approach has proven valuable in uncovering the physiological and medicinal potential of plant extracts. Qualitative phytochemical screening has been used to determine numerous secondary plant metabolites [54]. Through the phytochemical analysis conducted in the study, the crude extracts were found to contain various phytochemicals, including carbohydrates, reducing sugars, alkaloids, polyphenols, triterpenoids, cardiac glycosides, and flavonoids. The screening

also revealed the presence of phenolic compounds, which have been associated with various physiological activities such as anti-carcinogenic, anti-inflammatory, anti-aging, and anti-apoptotic activities, angiogenesis inhibition, and endothelial function enhancement [55].

In contemporary research, there has been a notable increase in the use of GC-MS analysis to identify chemical compounds in medicinal plants and traditional remedies. Metabolic flux analysis (MFA) is a technique that offers substantial insights into the distribution of isotopic isomers. Consequently, it has been employed to analyse compounds such as lipids, non-polar entities, volatile essential oils, and fatty acids [56]. A total of 81 components were detected in the extract, comprising multiple chemical compounds such as esters, fatty acids, phenols, and alcohols. The chemical extracted from MEFSF exhibits the potential for application as a novel pharmaceutical agent for treating various ailments, including cancer, depression, and neurodegenerative disorders.

The brine shrimp lethality bioassay is a cost-effective, efficient, and secure technique that is used to assess the bioactivity of plant extracts. A notable association exists between the lethality bioassay of brine shrimp and potential cytotoxic and pesticidal action against human malignancies. This association is valuable for developing effective natural pesticides and anti-tumour agents. In general, a higher LC_{50} peak value indicates lower toxicity, whereas a lower peak value indicates higher toxicity. During our initial cytotoxicity evaluation, we found that the LC_{50} value of MEFSF was 267.23 µg/mL, indicating a moderate level of toxicity. In contrast, vincristine sulphate had a notably elevated degree of toxicity, as indicated by its LC_{50} value of 2.15 µg/mL, which indicates that it has high toxicity.

Depression and anxiety are widely prevalent and incapacitating neuropsychiatric conditions, and stress plays a crucial role in their development [57]. Depression can manifest through several physiological symptoms, including decreased appetite, cognitive impairment, and disrupted sleep patterns [58]. Additionally, it is characterised by emotional manifestations such as apathy, despair, aboulomania, and diminished self-confidence. This neurodegenerative condition poses a significant hazard to individuals in terms of both their psychological and physical well-being. Nevertheless, previous studies have indicated that variations in the levels of corticotropin-releasing factor (CRF) in the cerebrospinal fluid may have distinct regulatory effects on depression and anxiety disorders [59]. Various antidepressants, including SNRIs and SSRIs, have been used to treat depression and anxiety disorders, with a declining success rate observed over time. The diminishing efficiency and safety profile of these drugs contribute to their lack of effectiveness. Researchers commonly prioritise the study of alternative approaches to identify more efficient and safer alternatives for managing depression. In recent years, medicinal plants have garnered significant attention owing to the potential therapeutic benefits of traditional medicines. Numerous phytochemicals, such as flavonoids, alkaloids, and terpenes, have demonstrated notable efficacy in combating depression when used as antidepressants [60].

In the assessments conducted in this study, we focused on measuring the duration of immobility—a key parameter that is indicative of depressive-like behaviour. Notably, MEFSF administration achieved a statistically significant ($p < 0.0001$) dose-dependent reduction in the immobility duration. This robust reduction highlights the antidepressant efficacy of MEFSF in both models. The most substantial reduction in immobility in mice was observed with a 400 mg/kg dose of the crude extract, with the immobility duration decreasing by 49.16% in the FST and 51.55% in the TST. These findings not only highlight the significant antidepressant potential of MEFSF but also suggest that it may hold promise as a therapeutic agent for depressive disorders. Researchers have demonstrated the existence of a neuropharmacological ‘halo effect’ where in, once anxiety is successfully treated, sadness may also be relieved [61]. However, when anxiety occurs repeatedly, it can turn into a severe psychological disorder [62]. Compared to exposure to an enclosed arm, approach-avoidance conflict was elicited when mice experienced significantly increased anxiety in the EPM test. Anxiogenic effects – demonstrated by more entries and longer durations spent in the open arm – reduce compared to the open arm [63,64]. In our investigation, we found that doses of 200 mg/kg and 400 mg/kg of the plant extract profoundly impacted the duration and frequency of the mice running in the open arms of the EPM test. Notably, a 400 mg/kg dose of MEFSF resulted in a significant increase in the number of entries (75.06% ± 2.79%) and the timespent in the open arms (78.39% ± 1.80%). These results indicate a statistically significant ($p < 0.005$) enhancement in exploratory behaviour in the anxiolytic situation created by the EPM. Similarly, the HBT evaluates the angiogenic behaviourof the mice and various unconditioned behavioural aspects in an unfamiliar environment [65]. A frequent tendency for hole poking (head dipping) indicates more anxiolytic activity, whereas a reluctance to visit a hole is considered a sign of worry [66]. Both the 200 mg/kg and 400 mg/kg doses of MEFSF resulted in significant ($p < 0.005$) increases in the exploratory behaviour of the mice, with a particularly notable increase with the 400 mg/kg dose. These improvements are characterised by a substantial increase in hole-poking behaviour, marked by a higher frequency of headdipping actions. These findings emphasise the potential of MEFSF as a cognitive enhancer, which may be linked to the presence of certain chemical substances that were measured and identified during the GC-MS analysis.

Diarrhoea is caused by ricinoleic acid, an active component of castor oil, which causes increased intestinal motility, electrolyte release, luminal osmolarity, and decreased electrolyte absorption [67,68]. The release of ri-

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cinoleic acid from castor oil by the lipase enzyme irritates the intestinal mucosa, and some inflammatory mediators like cyclic adenosine monophosphate, prostaglandin, and nitric oxide, tachykinins, and platelet-activating factor are secreted owing to the irritation. These inflammatory mediators promote intestinal motility and electrolyte and water increase. Ricinoleic acid activates the G-protein-coupled prostanoid receptor (EP3) on the intestinal smooth muscle cells and manifests diarrhoeal symptoms [69,70]. In our investigation, the crude extract exhibited significant efficacy in reducing motility by suppressing prostaglandin production in both anti-diarrhoeal models. Notably, the 400 mg/kg dose demonstrated a highly significant ($p < 0.0001$) level of inhibition that was comparable to that of the reference standard drug loperamide. This anti-diarrhoeal property of MEFSF is likely due to certain chemical compounds that were identified through GC-MS analysis.

The molecular docking process is a well-known structural drug development technique that is frequently used to create therapeutic agents for complicated disorders by simulating interactions between drug molecules and therapeutic targets, including enzymes and receptors [30]. The purpose of docking is to determine a ligand's orientation and binding strength within a protein binding site. It helps to rationalise and interpret the structure-activity relationships (SAR) between naturally occurring compounds [71]. In this study, we implemented an *in silico* molecular docking method to identify potential phytochemicals that may mechanistically contribute to the observed cytotoxic, neuropharmacological, and anti-diarrhoeal activities of MEFSF, as well as to validate and correlate our experimental findings at the molecular level. The docking experiment using four therapeutic target proteins yielded seven potential compounds from MEFSF that possess a high affinity against multiple targets. These compounds are beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl, 13-Docosenamide, (Z), Lup-20(29)-en-3-ol, acetate, (3.beta.), Urs-12-en-3-ol, acetate, (3.beta.), (-)-Globulol, Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl), and Thunbergol. Beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl was analysed to have a strong binding affinity against all target proteins. Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methyl phenyl), and Thunbergol also manifested a potent binding affinity against hER, K^+ channel, and M3MAR. These compounds may be directly responsible for the MEFSF's cytotoxic, anxiolytic, antidepressant, and anti-diarrhoeal activities, and these effects may be mediated in part by modulation of the activities of the K^+ channel, SERT3, and M3MAR, respectively.

Pharmacokinetics and toxicological characteristics are considered essential for identifying and advancing novel pharmaceutical compounds. The significant failure rate observed in clinical trials over the past few decades can be attributed to complications arising from the pharmacokinetics and toxicological profiles of drug candidates. This has impeded the market entry of several medications. In addition to examining pharmacological profiles, research has shown that properties related to absorption, distribution, metabolism, and excretion play a crucial role in determining the optimal therapeutic effectiveness of a drug. The ADME/T profiles of the seven highest-scoring compounds from the molecular docking process were examined in greater detail using the SwissADME and PkCSM online tools. Various absorption metrics, including the Caco-2 permeability and intestinal absorption, were assessed alongside the physicochemical properties indicated by Lipinski's and Veber's criteria to substantiate the oral bioavailability extent of these substances. The cumulative results suggest that six substances are expected to exhibit high absorption and bioavailability rates. During the assessment of the distribution characteristics, it was observed that these compounds have the capacity to traverse the central nervous system (CNS) and the blood-brain barrier (BBB). This ability is crucial for manifesting optimal neuropharmacological effects. In addition to satisfying most metabolism and excretion criteria, it is improbable that these chemicals would induce Ames toxicity, hepatotoxicity, or acute oral toxicity in mice. The results of this *in silico* investigation of the interactions between the putative bioactive compounds derived from MEFSF and specific target proteins can serve as an important guide for identifying and developing innovative therapeutic approaches for cancer, neuropsychiatric disorders, and anti-diarrhoeal action. However, this work primarily serves as an initial effort for understanding the pharmacological impacts of MEFSF and its possible bioactive metabolites by examining the correlation between the findings of experimental and computer-assisted models.

Conclusion

Based on the findings of this study, we infer that *F. semicordata* an potentially serve as a valuable source of phytochemicals that exhibit significant neuropharmacological and anti-diarrhoeal properties, with moderate cytotoxic effects. The GC-MS investigation revealed the presence of several bioactive compounds. Seven compounds exhibiting high affinities for multiple targets were discovered from a raw extract using computer-assisted analyses and molecular docking techniques involving four therapeutic target proteins. Further comprehensive *in vivo* and *in vitro* research is essential to identify and evaluate the lead molecules accountable for these biological potentials.

Availability of Data and Materials

This published paper comprises the data acquired or researched during this project. Information will be supplied upon proper request.

Author Contributions

All authors contributed to the study conception and design. MAM, FIF, MAT and JHA designed the study. MAM, FIF, MAU, MNI, MAS, MZU and TBE help in data curation. MAM, FIF, MAT, MNI, HAA-M, SFA, RB and TBE help in formal analysis. HAA-M, SFA and TBE help in funding acquisition. MNI, MAS, SFA, RB and TBE provide the resources/software. MAM, FIF, MAT, JHA, MNI, HAA-M, SFA and RB perform the initial drafting. MNI, FIF, MAS, MZU, RB and TBE help in review and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The P&D committee (Pharm-P&D17/08'-19), Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh, sanctioned & authenticated the whole study protocol. According to the reference number IIUC/PHARM-AEC-150/20-2019, every experimental protocol that is concerned with this research was approved by the IAEC (Institutional Animal Ethical Committee), Department of Pharmacy, International Islamic University Chittagong, Chittagong-4318, Bangladesh. In this report, all the sections were conducted through the guidelines of "Animal Research: Reporting of *In Vivo* Experiments" and the "Principles of the Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) and "National Animal Care Laws" were strictly imitated in time of animals handling.

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Conflict of Interest

The authors declare no conflict of interest. Talha Bin Emran is serving as one of the Guest editors of this journal. We declare that Talha Bin Emran had no involvement in the peer review of this article and has no access to information regarding its peer review.

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