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The cytotoxic and anti-depressant activity of ethanol extract of *Pterospermum semisaggitatum* (buch. – ham.exroxb.) leaves: an *in vitro* and *in vivo* study

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Abstract

This report investigates the phytochemical, cytotoxic and antidepressant property of the ethanolic extract of *Pterospermum semisagittatum* from South Asia region. Extracts subjected to pharmacological activity using *in vitro* and *in vivo* methods which are cytotoxic and antidepressants activity on animal model alongside its phytochemical profiling. The cytotoxic activity was investigated using brine shrimp lethality assay and antidepressant activity was determined by forced swimming test (FST) and tail suspension test (TST) in mice. The plant extract of ethanol extract of *Pterospermum semisagittatum* (EEPS) was found secondary metabolites, notably steroids, glycosides, tannins, flavonoids, saponins, and alkaloids etc. EEPS showed substantial cytotoxic effects lethal concentrations (LC_{50}) value are 347.65 µg/mL. Besides, treatment with EEPS revealed a significant reduction of immobility time in a dose-dependent manner in Force Swimming Test (FST) and Tail Suspension Test (TST). The dose of 400 mg/kg, body weight shows the best antidepressant effects in both FST and TST which are respectively immobile time 198.5± 3.81**** and 157.5 ± 4.01**** statistically significant (****P < 0.0001). Subsequently, the dose of 200 mg/kg, body weight shows the best antidepressant effects in both FST and TST which are respectively immobile time 180± 3.03**** and 33.75± 2.95**** (statistically significant).The investigation concludes that EEPS can be a potent source of antidepressant and cytotoxic agents.

Keywords: Ethanol; *Pterospermum semisagittatum*; phytochemicals; cytotoxic and antidepressant.

Introduction

The biological resources found in nature, such as plants, animals, and microbes, can be utilized for various purposes such as medicinal, social, and environmental goals. There are a vast number of medicinal plants around the world that exhibit diverse pharmacological effects. These medicinal herbs are a valuable gift from nature that can aid us in living a healthy and illness-free life. (Akanda & Hasan, 2021; Alam et al., 2021; Arman et al., 2022). In order to help people live a healthy, disease-free life, medicinal plants are nature's gift to people (Parves, 2016). For millennia, medicinal plants have been used, and their bioactive natural compounds play a significant role in safeguarding health in both the pharmaceutical and food industries, as well as contributing to the fragrance, agricultural, and personal-care product sectors (Rasool,2012;Guha et al., 2021). Approximately 70% of new pharmaceutical compounds and their derivatives are derived from plants (Du & Tang, 2014). Complementary and alternative medicine (CAM) encompasses a broad spectrum of diagnostic and therapeutic techniques in dermatology that complement conventional

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dermatological procedures, incorporating the latest scientific advancements and CAM practices. (Tirant et al., 2018). Around 80% of the global population uses traditional botanical remedies, according to the World Health Organization (WHO), with 40,000 to 70,000 species of medicinal plants used as traditional remedies across the globe(Hosseinzadeh et al., 2015; Verpoorte et al., 2006). Discovering lead compounds that possess the necessary biological activity is an essential part of creating a new medication. These lead compounds, which can have various beneficial effects such as fighting cancer, bacteria, reducing pain, relieving diarrhea, lowering blood sugar, and acting as antidepressants for the central nervous system, are already present in nature. (Islam et al., 2019). The evaluation of the crude plant extract for its purported medicinal benefits often occurs at the beginning of the investigation for the discovery of bioactive components (Chandrakant et al., 2012). In Malaysia, the plant grows abundantly and adds to the beauty of the parks and roadsides, making it an attractive sight. Throughout history, it has been believed that medicinal herbs are a vital source of sustenance for human beings. Utilizing plants and natural products derived from plants is essential for promoting good health due to their rich variety of nutritional benefits. These include vitamins, minerals, phenolic compounds, fiber, antioxidants, and bioactive metabolites, all crucial for maintaining well-being. (Khan et al., 2020).

Phytochemicals produced by plants are classified into two main categories, primary and secondary metabolites. Primary metabolism involves the creation and breakdown of essential substances such as proteins, lipids, nucleic acids, and carbohydrates, which are necessary for all living organisms. The substances involved in these metabolic pathways are known as primary metabolites(Velu et al., 2018). The production of secondary metabolites or natural products by an organism is a process that is often unique to the organism or is a reflection of the species' distinct characteristics. Unlike primary metabolites, secondary metabolites are not usually essential for an organism's growth, development, or reproduction. Instead, they are created as a result of the organism adapting to its environment or as a potential defense mechanism against predators, aiding in the organism's survival (Velu et al., 2018; Dhaniaputriet al., 2022). According to Harborne and Baxter (Harborne et at., 1993), the primary aim of qualitative phytochemical analysis is to provide a preliminary assessment of the presence or absence of certain classes of compounds in plant extracts such as alkaloids, flavonoids, saponin, tannin etc. These substances are recognized for their pivotal role in the medicinal and therapeutic attributes exhibited by plants. Therefore, the identification of these bioactive compounds is necessary to ensure the efficacy and safety of medicinal plant products.

Cytotoxicity tests are important methods for evaluating the potential toxicity of compounds and extracts from medicinal plants. These tests are commonly used in drug discovery and development to identify compounds with potential anticancer and antiproliferative activity. One common cytotoxicity assay is the brine shrimp lethality assay, which has been used for preliminary screening of potential anticancer and antiproliferative agents due to its simplicity, cost-effectiveness, and ability to test large numbers of samples in a short period of time (Mosmann,1983). Additional frequently employed cytotoxicity assessments comprise the MTT assay, gauging cellular mitochondrial activity, and the lactate dehydrogenase (LDH) assay, quantifying the discharge of lactate dehydrogenase stemming from cell injury. These assays are more advanced and are often used to confirm the results of brine shrimp lethality assays (Van Meerloo et al., 2011).

Neurodegenerative diseases are a set of long-term and progressive ailments that impact the central nervous system, causing deterioration in cognitive ability, motor

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10.5530/ctbp.2024.3s.15

functions, and behavior. The incidence of neurodegenerative diseases is on the rise worldwide, with estimates suggesting that the number of people affected by these disorders will triple by 2050 (Pringsheim et al., 2014). Neurodegenerative diseases are complex and progressive disorders affecting the central nervous system, resulting in cognitive and motor function decline. Polyphenols, alkaloids, terpenoids, and flavonoids are among the phytochemicals with neuroprotective effects by modulating pathways related to oxidative stress, inflammation, and protein aggregation (Shallie, 2020). Antidepressants are pharmaceuticals employed in the management of diverse mental health issues like depression syndromes, anxiety like disorders, and OCD(Obsessive-Compulsive-Disorder). They function by modifying the concentrations of neurotransmitters within the brain, which play a pivotal role in regulating mood, behavior, and emotions. Tricyclic antidepressants (TCA), monoamine oxidase inhibitors (MAO inhibitors), selective serotonin reuptake inhibitors (SSRI), serotonin antagonist and reuptake inhibitors, norepinephrine and dopamine reuptake inhibitors, and serotonin and norepinephrine reuptake inhibitors are all indispensable players in the diverse pharmacological arsenal deployed to combat depressive disorders (Arroll et al., 2016). The flora for example *Pterospermum semisagittatum* is naturally occurring in Asia. This plant is typically employed as complementary therapies to treat ailments including malignancies, tumors, cardiac palpitations, sensations of burning, hepatic ailments digestive problems, respiratory tract disorders, skin disorders, malaria, as well as rheumatic pain (Taraquzzaman et al., 2014). The aim of this investigation was to delve into the ingenious crafting of groundbreaking bioactive compounds derived from the wondrous *Pterospermum semisagittatum*. These compounds were tailored with the captivating goal of significantly mitigating toxicity while fostering remarkable recuperation from various disorders.

Materials and methods

Drugs and chemicals

The substances used in the research included ethanol (R & M Chemicals, Selangor, Malaysia). Fluoxetine (Eskayef Banlgadesh Ltd, Bangladesh) and the remaining chemicals were purchased from a local vendor through Chemiz Ltd in Malaysia.

Collection, identification of plant and extraction

Fresh *Pterospermum semisagittatum* leaves were collectedlocally from the Putrajaya, Malaysia. The plants were subsequently identified by Dr. Khairil Mahmud, the Biodiversity unit at the Institute of Bioscience at the University of Putra Malaysia, as well as Dr. Nor Azam Bahari, the attending veterinarian at the same institution. After being shade-dried, the dried leaves were then crushed into the form of powder by means of a mechanical crusher (Sieve No. 10/44). Subsequently, the powder had been extracted for seven consecutive days in a row by using laboratory-grade ethanol and a Soxhlet apparatus (Dhaniaputri et al., 2022). Concentrated crude ethanol extract (EEPS) was prepared by evaporating the resulting extracts by means of a Rotavapor (Buchi Flawil, Switzerland) at reduced pressure. The extract had been preserved cooled till it was required for the purpose of the investigation.

Experimental design

To perform the in vivo study, twelve mice were separated into four groups (Group I-IV), with each group consisting of three animals (n = 3). The therapy procedure was developed as follows: Group received the vehicle (1% tween 80 in distilled water, 10 mL/kg b.w., p.o.), Group received the standard medicine (Fluoxetine HCl 20 mg/kg b.w., p.o.), and Groups and IV received EEPS 200 and 400 mg/kg b.w., p.o. respectively.

Vol. 18 (Supplementry Issue 3s) 200 - 208, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
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Qualitative phytochemicals study

The qualitative phytochemical study was analyzed using standard protocols (Khan et al., 2020) which involved checking for the presence of secondary metabolites such as flavonoids, alkaloids, carbohydrates, protein & amino acid, saponins, glycosides, steroids, tannins, phenols, cholesterols, resins and reducing sugar (Table 1).

Cytotoxic assay by brine shrimp lethality bioassay

To determine cytotoxic action, crude extracts of *P. semisagittatum* leaves were tested using a brine shrimp mortality bioassay. The process commenced with the careful incubation of Artemia salina, more commonly known as brine shrimp eggs, within a specialized water reservoir enriched with sea salt. Within a cozy environment maintained at a temperature range of 22-29°C, the tiny nauplii emerged, drawn towards a radiant light source positioned strategically on one side of the vessel. These nascent organisms were then delicately harvested and injected into miniature buckets brimming with pristine seawater, a process repeated 2-3 times to ensure optimal cultivation. The test dosage was gradually increased to 800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/ml. Vincristine and DMSO were used as positive and negative controls, respectively, to compare to the test group (Pisutthanan et al., 2004;Rahman et al., 2013). This finding was used to determine the percentage of mortality in brine shrimp nauplii at different saturation level.

Forced swimming test (FST)

The FST method proved efficacious in evaluating the antidepressant impact on

Table 1: Phytochemical screening of ethanol extract of *P. semisagittatum* leaves

Sl.	Phytochemicals	Name of the tests	Observation
1	Flavonoids	Wagner's reagent test	+
2	Alkaloids	Wagner's reagent test	+
3	Carbohydrates	Benedict test	+
4	Protein & amino acid	Sulphuric acid test	+
5	Saponins	Foam height test	-
6	Glycosides	Keller-killiani test	+
7	Steroids	Salkowski test	-
8	Tannins	Lead acetate test	+
9	Phenols	Iodine test	+
10	Cholesterols	Salkowski test	+
11	Resins		-
12	Reducing sugar	Benedict test	+

(+) means present, (-) means absent

Values have been presented as mean ± SEM (n = 3) with the P value ($^{****}P < 0.0001$) being statistically significant in comparison to the control group ($P > 0.05$) processed by Dennett's test by using one-way ANOVA analysis (graph pad prism software, version 8.4.4) for multiple comparisons. EEPS = Ethanolic extract of *Pterospermum semisagittatum*

Vol. 18 (Supplementry Issue 3s) 200 - 208, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.15

Swiss albino mice. According to the requirements of the research design, the therapy was delivered a half-hour before to the experiment. The experimental mice had been kept individually in an open cylindrical box with a 19 cm depth of water at a constant temperature of 25 °C (10 cm height x25cm diameter). After using each mouse to execute an FST, the water within the compartment changed since "used water" was utilized to change the games. Within the first two minutes of the test, every mouse displayed complete mobility at some point. At some point during the following four minutes of the total six minutes of checking out time, the period of inactivity was manually recorded. Mice were deemed immobile if they remained afloat without exhibiting any movement beyond what was essential to maintain their heads above the water. Mice in each of the three groups received the same care (Islam et al., 2021; Khan et al., 2020).

Tail Suspension Test (TST)

To assess the behavioral effects of antidepressants on mice, the TST was employed. According to the research strategy, the therapy was administered 60 minutes prior to the start of the experiment. In order to determine the total length of immobility brought on by tail suspension, mice were suspended 50 cm above the ground, positioned on the table's edge, with adhesive tape securely affixed just 1 cm from the tip of each mouse's tail.. Each mouse from all agencies was timed over the final four minutes of a six-minute period. An animal hanging passively and without motion ceased to exhibit any frame motion when it was measured to be motionless. Mice in each group received the same care (Hossen et al., 2021; Vickers, 2017).

Statistical analysis

The values were showed in mean ± standard error mean (SEM). $P < 0.05$statistically significant, which was carried by one-way ANOVA (Dunnett's test) using GraphPad Prism (version 8.4.) software.

Results

Qualitative phytochemicals study

The qualitative analysis of phytochemicals in this study indicated the presence of flavonoids, alkaloids, carbohydrates, proteins & amino acids, glycosides, tannins, phenols, cholesterols, and reducing sugars.

Cytotoxic assay by brine shrimp lethality bioassay

Brine shrimp lethality activity of the ethanolic extracts of *P. semisagittatum*was presented in (Figure 1). The crude extract showed 75% mortality at 800 µg/mL concentration and LC_{50} value was 347.65µg/mL which was were considered significantly active. No mortality was found in negative control (DMSO) group.

Effects of EEPS on FST and TST

Figures 2 and 3 illustrate the effects of oral administration of the EEPS solution on immobility time in the FST and TST respectively. As depicted in figures, the extract was given by oral route at doses of 200 and 400 mg/kg significantly ($^{****}P < 0.0001$) decreased the immobility time in both TST whereas 200 and 400 mg/kg revealed significant ($^{****}P < 0.0001$ and $^{***}P < 0.001$) decrease in a dose-dependent manner as compared to the control group. In order to rule out the possibility that the extract's anti-

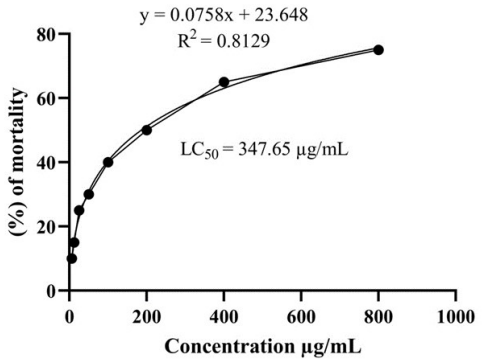

Figure 1: Cytotoxicity activity of EEPS

The cytotoxic and anti-depressant activity of ethanol extract

Vol. 18 (Supplementry Issue 3s) 200 - 208, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.15

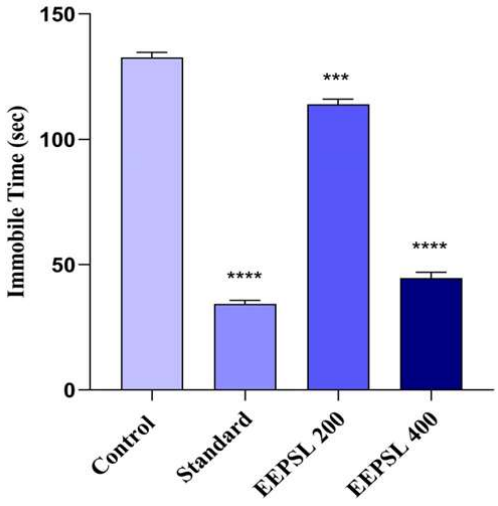

Figure 2: Antidepressant activity of EEPS through Forced swim test (FST) test in *Swiss albino* mice

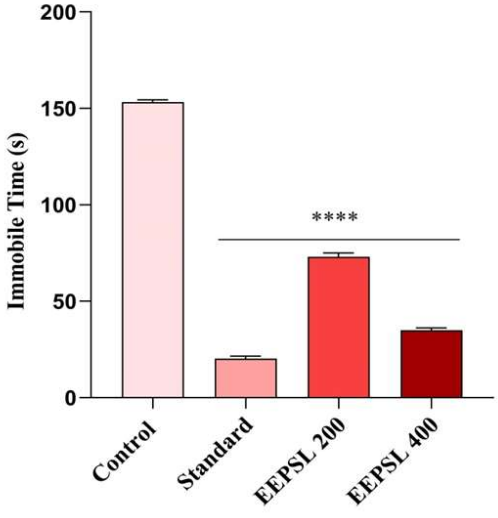

Figure 3: Antidepressant activity of EEPS through Forced swim test (TST) test in *Swiss albino* mice

immobility effect was attributable to a psychostimulant impact, the dosages that generated an effect in the FST were given to a separate group of mice who were then evaluated in the TST.

Discussion

Phytochemical screening is an important element of plant-based research since it detects the existence of various chemical substances that may have therapeutic advantages. The EEPS was subjected to a qualitative phytochemical study, which revealed varied quantities of carbohydrates, alkaloids, steroids, glycosides, tannins, flavonoids, saponins, terpenoids, and phenols. The plant's biological effects might vary depending on the phytochemicals present (Khyade et al., 2014).

Several studies have demonstrated the usefulness of the Brine Shrimp Lethality Assay(BSLA) in screening for the toxicity of different compounds. For example, researchers have used this assay to evaluate the toxicity of plant extracts (2), marine natural products (3), and various pharmaceuticals (4) (McLaughlinet et al., 1998). One advantage of the BSLA is that it can be used to screen a large number of compounds rapidly and inexpensively. Moreover, the BSLA is a relatively simple assay that requires minimal equipment and expertise. However, it is important to note that the BSLA is a preliminary screening tool and that further testing, such as in vivo studies, is necessary to confirm the toxicity of the compounds identified in this assay. By plotting the proportion of shrimp deaths against the logarithm of the sample concentration (toxicant concentration), the lethal concentration (LC_{50}) of the test samples was determined after 24 hours. The best-fit line was then determined from the curve data using regression analysis.

Forced Swim test involves placing a mouse in a container of water from which it cannot escape, and measuring the time the mouse spends immobile or struggling to escape over a defined period of time. Several studies have used the FST to evaluate the antidepressant activity of various drugs, including selective serotonin reuptake

Vol. 18 (Supplementry Issue 3s) 200 - 208, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.15

inhibitors (SSRIs), tricyclic antidepressants (TCAs), and monoamine oxidase inhibitors (MAOIs). For example, a study by *Porsolt et al*. (Porsolt et al., 1997) showed that acute treatment with the SSRI fluoxetine reduced immobility time in mice, indicating antidepressant activity.However, there are also criticisms of the FST as a measure of antidepressant activity. Some researchers argue that the test is highly influenced by non-specific factors such as stress and fatigue, and that it does not necessarily reflect the clinical efficacy of antidepressants in humans (Cryan et al., 2005). High rates of chronicity, recurrence, and suicide are all related with the serious but common condition of depression. Although there are numerous antidepressant medications available, their use is sometimes accompanied by unpleasant side effects, and some depression patients have a limited response to their therapeutic benefits (Kwon et al., 2010). The EEPS demonstrated a clear antidepressant-like effect in both the forced swimming and tail suspension tests in the current study. The immobility of mice occurs in a condition of despair or reduced mood, which is similar to human depression (Foyet et al., 2011). The mice had given up on the idea of escaping the restricted region. It has been shown that antidepressant medications can shorten the length of immobility in the animal model (Abelaira et al., 2013). Antidepressants activity of plant extracts may be due to the presents of alkaloid, carbohydrate, flavonoid and saponin (Bahramsoltani et al., 2015) and the presents of alkaloids, carbohydrates, flavonoids and saponin represents the function of EEPS.

Conclusion

Finally, the study underscores *Pterospermum semisagittatum's* potential as a source of bioactive chemicals having pharmacological properties, such as cytotoxicity and antidepressant effects. The presence of phenolic and flavonol components in the ethanolic extract of *P. semisagittatum* indicates that these phytochemicals may be responsible for the observed bioactivity. The findings of this study provide a foundation for further research into the active compounds' mechanisms of action and potential as lead molecules for medication development. Natural products, such as *P. semisagittatum,* can be used to generate novel medications that are safer and more effective than traditional pharmaceuticals.

Contributions

MHU and IKM designed the experiments, MHU carried out the experiments, MHU has written the initial draft. MHU and IKM, refined the manuscript. All authors have read and approved the manuscript.

Ethics approval

Cyberjaya University Animal Care & Use Committee (CACUC), wide reference number CACUC/1/2023/3.

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Vol. 18 (Supplementry Issue 3s) 200 - 208, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
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