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ANXIOLYTIC, HYPNOTIC AND ANTI-SEIZURE ACTIVITIES OF *SONNERATIA APETALA* (BUCH.-HAM.) FRUIT THROUGH THE $GABA_A$ERGIC AND OPIOIDERGIC RECEPTORS

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Abstract

The study evaluated the extract of the fruits of *Sonneratia apetala* (Buch.-Ham.) for the treatment of anxiety, insomnia, and seizures. For this, the fruits were successively extracted with *n*-hexane (Hex), chloroform (Chl), and methanol (Met). All the extracts of the fruit showed an anxiolytic-like ($p < 0.05$ vs. control) effect in mice, as did diazepam (Dzp), with Hex showing the highest, followed by Met and Chl, in the elevated plus maze test. Flumazenil (Flu), a $GABA_A$ receptor antagonist, and naloxone (Nal), an opioid receptor antagonist, inhibited the anxiolytic effects of Hex and Met in mice. In the thiopental-induced mice model, Hex and Met extracts significantly shortened the latency time and extended the duration time of sleep compared to the control (C) mice. Moreover, Hex and Met extracts significantly delayed the appearance of the characteristic tonic-clonic seizures in the pentamethylenetetrazole-induced mice model. Therefore, *S. apetala* fruit possesses anxiolytic, hypnotic, and anti-seizure properties, which modulate the $GABA_A$ and opioid receptors.

Introduction

Anxiety is a psychoneurophysiological state that defines the behavioral patterns of an individual towards stress. Worldwide, 301 million people have anxiety disorders, and among them, women are 1.66 times more to men (Javaid *et al.* 2023). Though various groups of drugs are used to treat anxiety disorders, benzodiazepines are the most common. Benzodiazepines bind with the interface of α and γ subunits of $GABA_A$ receptors (Sigel and Steinmann 2012) in the central nervous system of the brain. Moreover, serotonin, dopamine, noradren (Vismara *et al.* 2020), and opioid (Colasanti *et al.* 2011) neuroreceptors are also involved in the manifestation of anxiety disorders.

Anxiety, insomnia, and epileptic seizure disorders affect 4.05% (Javaid *et al.* 2023), 8.5 to 23.6% (Porcheret *et al.* 2024), and nearly 1% (Walton *et al.* 2021), respectively, in the population worldwide. However, the drugs used to treat these diseases, including benzodiazepines and antidepressants, cause various side effects- sedation, drowsiness, dizziness, nausea, muscle relaxation, amnesia, and physical dependence (Longo and Johnson 2000, Kaplan and Sadock 2005). Therefore, to prevent or treat these diseases, new alternative approaches based on dietary interventions with fewer unwanted effects have been identified. Alramadhan *et al.* (2012) mentioned several nutrients and botanicals to reduce anxiety. Edible fruits such as noni (Deng *et al.* 2007), passion fruit (Deng *et al.* 2010), blackberry (Fernandez-Demeneghi *et al.* 2019), and beverages such as tea and coffee (Hossain *et al.* 2003, 2004, 2007) showed anxiolytic, sedative, and anticonvulsive effects.

Among the edible fruits in the Sundarbans' mangrove forest, the fruit of *S. apetala*, locally known as Keora fruit, is extensively consumed by coastal Bangladesh, India, Sri Lanka, Myanmar etc. Previous reports described the fruit as a potential source of nutrients, polyphenols, flavonoids, vitamin C, and antioxidants with anti-diabetic, antibacterial, antidiarrheal, analgesic, and iron-

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chelating properties (Hossain *et al.* 2013, 2016, 2017; Mithila *et al.* 2023). Moreover, the fruit protected against acetaminophen-induced liver injury in mice (Liu *et al.* 2019); extended the lifespan in *Caenorhabditis elegans* (Jiang *et al.* 2022); and inhibited elastase activity to prevent lung injury (Sengupta *et al.* 2022). Though the fruit of *S. apetala* is composed of high functional components, no report described its anxiolytic, hypnotic, and anti-seizure activities; hence, the study was undertaken.

Materials and Methods

Fruits of *Sonneratia apetala* (Buch.-Hum.) were collected from the Sundarbans' mangrove forest of Bangladesh in September 2023. After washing, they were shade-dried and ground into a fine powder. The powder was successively extracted with *n*-hexane (Hex), chloroform (Chl), and methanol (Met). Briefly, 10 g of powder was extracted with 200 ml Hex at 30°C with frequent shaking for 3 days. The extract was filtered through Whatman no. 1 filter paper, and the filtrate was air-dried, weighed, and stored at 4°C as the Hex extract. The residue on the filter paper was used for preparing Chl and Met extracts, respectively.

Male Swiss-albino mice (18~22 g) were purchased from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR'B). Guidelines of the Animal Ethics Committee of Khulna University were strictly followed in conducting the experiments (Research ref. no. KUAEC-2022/09/17). The elevated plus-maze (EPM) test was used to assess the anxiolytic activity of the extracts. The percentage (%) of entry numbers and time spent in open or closed arms of EPM were used to measure the anxiolytic activity (Komada *et al.* 2008) in mice. To evaluate the possible involvement of the GABAergic and opioidergic receptors in the anxiolytic-like activities, mice-groups were also treated with flumazenil (10 mg/kg body weight, b.w., intraperitoneal, ip) and naloxone (3 mg/kg b.w., ip), respectively, 15 min before the treatments with the potential extracts and standard drugs.

The thiopental-induced sleeping test in mice was performed for the hypnotic effects of the fractions. Thirty min after the treatments, mice were administered (ip) sodium thiopental at 30 mg/kg b.w. to induce sleep. Then, they were immediately observed for latency time (time required for the onset of sleep, which was indicated by the loss of righting reflex) and duration time (reappearance of righting reflex) of sleep.

Pentamethylenetetrazole (PTZ)-induced seizure was studied on mice (Chen *et al.* 2016). Sixty min after the treatments, an injection (ip) of PTZ at 80 mg/kg b.w. was performed. Immediately, time (sec) was counted by a stopwatch to know the latency period for the development of the characteristic tonic-clonic seizures.

The results were presented as mean ± SEM (n = 6). The data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post hoc test. Statistical significance was set at p-value < 0.05.

Results and Discussion

Oral administration of Hex, Chl, and Met extracts of the fruits of *S. apetala* at 100 and 500 mg/kg b.w., and diazepam (Dzp) at 2 mg/kg b.w. significantly ($p < 0.05$) increased the % open arms entries (% OAE), and % open arms time (% OAT) spent than that of control group mice (C) in the EPM (Fig. 1A and B), whereas inverse results were observed for closed arms. Among the extracts, Hex and Met showed similar effects as recorded from Dzp, while Chl exhibited a slightly smaller effect. It was proved that Dzp, an anxiolytic drug, increased % OAE and % OAT in the EPM test (Komada *et al.* 2008). Similarly, in this experiment, Dzp also increased % OAE and %

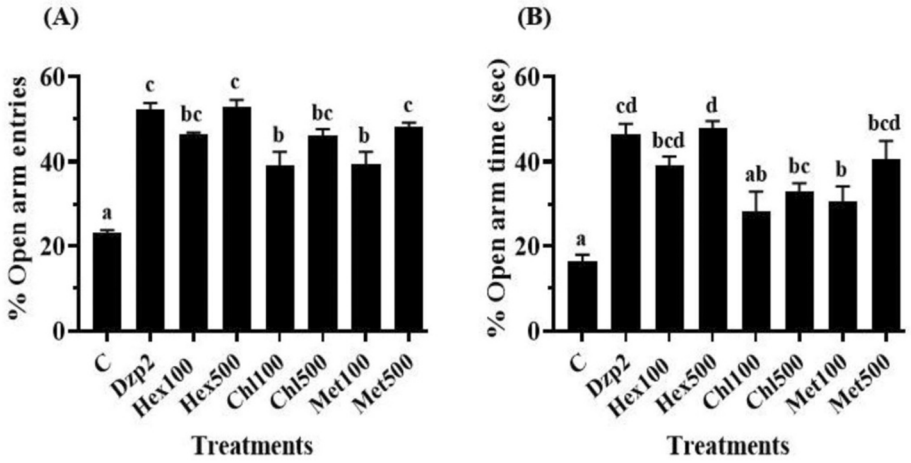

Fig 1. Anxiolytic-like activities of the *n*-hexane (Hex), chloroform (Chl), and methanol (Met) extracts of *Sonneratia apetala* fruit, and diazepam in mice (A, B). Percentage (%) open arm entries (A) and open arm time (B) in the elevated plus-maze (EPM) test. Mice were treated 0.9% saline water with Tween 80 (C, control, oral); diazepam (Dzp2, 2 mg/kg b.w., oral); Hex100, Chl100, and Met100 (100 mg/kg b.w., each extract, oral); and Hex500, Chl500, and Met500 (500 mg/kg b.w., each extract, oral) at 1 h before the EPM test. Data were presented as mean ± SEM, and the number of observations, n = 6. Different letters (a-d) indicated significant differences at $p < 0.05$.

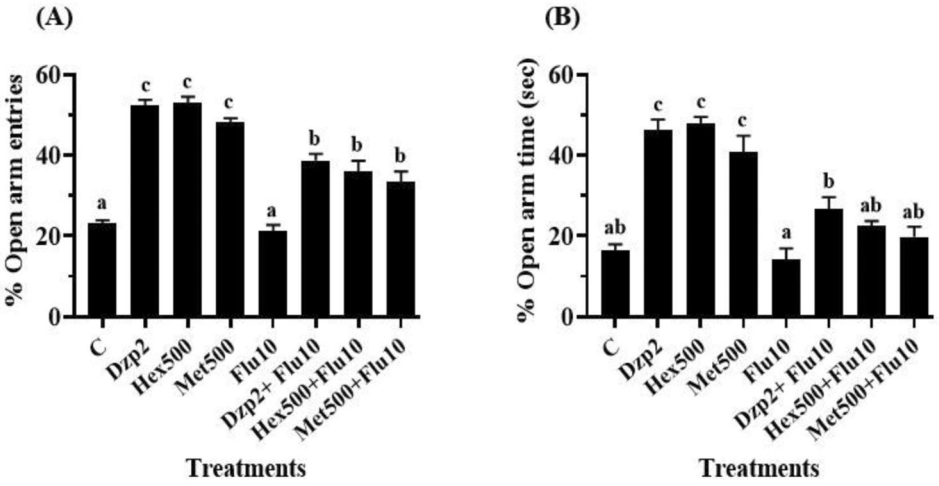

Fig 2. The effect of flumazenil on anxiolytic activities of the potential extracts [*n*-hexane (Hex), and methanol (Met)] of *Sonneratia apetala* fruit, and diazepam in mice (A, B). Percentage (%) open arm entries (A) and open arm time (B) in the elevated plus-maze (EPM) test. Mice were treated 0.9% saline water with Tween 80 (C, control, oral); diazepam (Dzp2, 2 mg/kg b.w., oral); Hex500, and Met500 (500 mg/kg b.w., each extract, oral); and flumazenil (Flu10, 10 mg/kg b.w., ip) at 1 h before the EPM test. Three groups of mice received Flu10 at 15 min before the treatments with Dzp2, Hex500, and Met500. Data were presented as mean ± SEM, and the number of observations, n = 6. Different letters (a-c) indicated significant differences at $p < 0.05$.

OAT to 52.3 and 46.3%, respectively, in the EPM test in mice. Therefore, the results demonstrated that all three extracts of *S. apetala* fruit had anxiolytic-like activity in mice. It is well-defined that flumazenil competitively binds with the benzodiazepine (i.e. diazepam) binding site on the $GABA_A$ receptors, and thereby antagonises the benzodiazepines' actions. When flumazenil (10

mg/kg b.w.) was administered (ip) 15 min before the oral treatment with Hex, and Met at 500 mg/kg b.w., and Dzp at 2 mg/kg b.w., it significantly antagonised their effects (Fig. 2A and B). Diazepam (Dzp), Hex, and Met showed % OAE of 52.3, 53, and 48%, and % OAT of 46.3, 47.8, and 40.7%, whereas administration of flumazenil decreased these % OAE to 38.7, 36, 33.5%, and % OAT to 26.5, 22.6, and 19.8%, respectively. Therefore, the compound(s) in Hex and Met extracts competed with flumazenil to bind to the Dzp binding site at the interface of α and γ subunits of the $GABA_A$ receptors (Sigel and Steinmann 2012). Hence, the anxiolytic-like effects of Hex and Met extracts of *S. apetala* fruit were mediated through the $GABA_A$ receptors.

Opioid receptors are the major components in the opioidergic system, involved in pain perception, reward, emotion, food intake etc. Fig. 3A and B showed that administration of Hex, Met, and morphine (Mor) in mice significantly increased % OAE of 57.9, 49.8, and 43.3%, and % OAT of 41.4, 44.3, and 33.4%, respectively, than that of control (C; % OAE 25.5; % OAT 18.2). Naloxone (Nal), an opioid receptor antagonist, when administered alone in mice, showed non-significant activity in EPM with the control (C) group. Conversely, when naloxone (3 mg/kg b.w.) was injected (ip) 15 min before the administration of Mor, Hex, and Met, significant inhibitions in % OAE and % OAT were observed. These results demonstrated that anxiolytic-like effects of the Hex and the Met extracts of *S. apetala* fruit were mediated through the opioidergic receptors. In previous studies, Mor was also reported as an anxiolytic drug because of increasing % OAE and % OAT in the EPM test (Babapoor-Farrokhran *et al.* 2008).

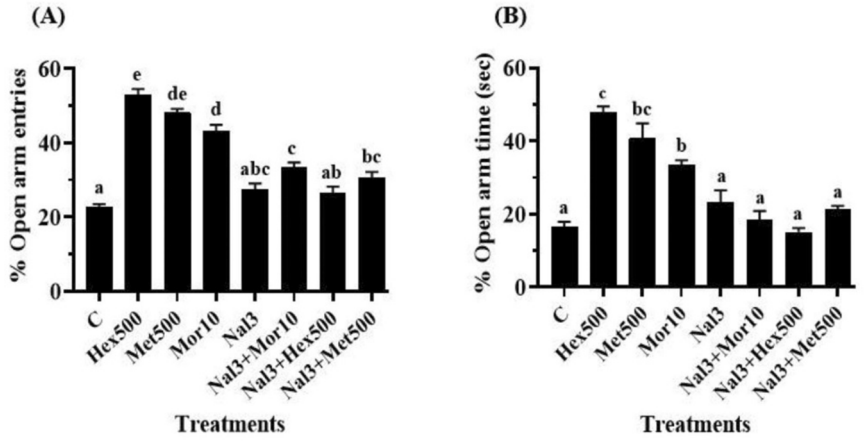

Fig 3. The effect of naloxone on anxiolytic activities of the potential extracts [*n*-hexane (Hex), and methanol (Met)] of *Sonneratia apetala* fruit, and morphine in mice. Percentage (%) open arm entries (A) and open arm time (B) in the elevated plus-maze (EPM) test. Mice were treated 0.9% saline water with Tween 80 (C, control, oral); morphine (Mor10, 10 mg/kg b.w., ip); Hex500, and Met500 (500 mg/kg b.w., each extract, oral); and naloxone (Nal3, 3 mg/kg b.w., ip) at 1 h before the EPM test. Three groups of mice received Nal3 at 15 min before the treatments with Mor10, Hex500, and Met500. Data were presented as mean ± SEM, and the number of observations, n = 6. Different letters (a-d) indicated significant differences at $p < 0.05$.

Thiopental is a derivative of barbiturates that acts as an agonist on $GABA_A$ receptors. It is used to induce sedation, hypnosis, and anesthesia because of its central nervous system depression effects. Intraperitoneal (ip) administration of thiopental (30 mg/kg b.w.) induced sleep in mice. Therefore, the latency time and the duration time of sleep in mice were determined. Mice treated with Mor at 10 mg/kg b.w. required the lowest latency time (1.9 min), followed by Dzp (2.8 min) for the onset of sleep, whereas that for control (C) was the largest (4.3 min). Hex and Met extracts

also needed significantly smaller latency time than the C (Fig. 4A). Duration time of sleep was largest for mice treated with Dzp, followed by Hex, Mor, and Met, whereas smallest for the C (Fig. 4B). Since Hex and Met extracts of *S. apetala* fruit significantly needed smaller latency time and larger duration time of sleep than the control mice, they had hypnotic properties. The compound(s) in the Hex and Met extracts of *S. apetala* fruit modulated $GABA_A$- (Fig. 2) and opioid- (Fig. 3) receptors, which could facilitate the thiopental-induced activation of inhibitory $GABA_A$ergic neurotransmission in mice. Potentiation of the recombinant $GABA_A$ receptors and pentobarbital-induced sleeping were also reported from fruits and medicinal plants (Mubassara *et al.* 2009).

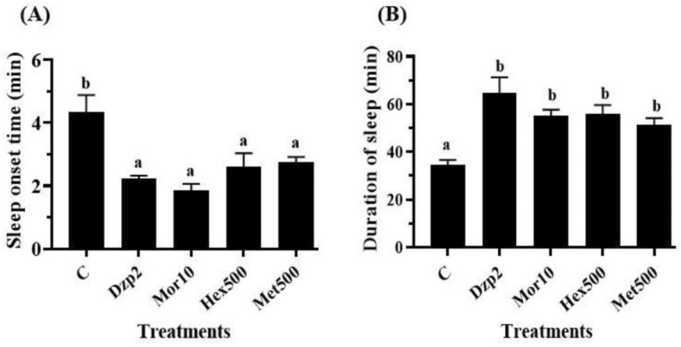

Fig. 4. Effects of the potential extracts [*n*-hexane (Hex), and methanol (Met)] of *Sonneratia apetala* fruit, diazepam, and morphine on the onset time (A) and the duration time (B) of sleep in thiopental-induced sleeping in mice. Mice were treated 0.9% saline water with Tween 80 (C, control, oral); diazepam (Dzp2, 2 mg/kg b.w., oral); morphine (Mor10, 10 mg/kg b.w., ip); Hex500, and Met500 (500 mg/kg b.w., each extract, oral) at 30 min before the treatment with thiopental (30 mg/kg b.w., ip). Data were presented as mean ± SEM, and the number of observations, n = 6. Different letters (a, b) indicated significant differences at $p < 0.05$.

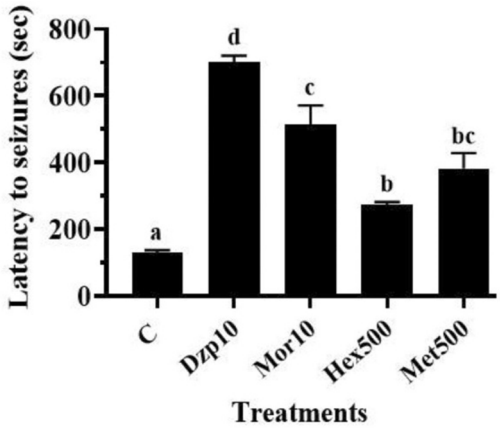

Fig. 5. Effects of the potential extracts [*n*-hexane (Hex), and methanol (Met)] of *Sonneratia apetala* fruit, diazepam, and morphine on pentamethylenetetrazole (PTZ)-induced tonic-clonic seizures in mice. Mice were treated 0.9% saline water with Tween 80 (C, control, oral); diazepam (Dzp10, 10 mg/kg b.w., oral); morphine (Mor10, 10 mg/kg b.w., ip); Hex500, and Met500 (500 mg/kg b.w., each extract, oral) at 1 h before the treatment with PTZ (80 mg/kg b.w., ip). Data were presented as mean ± SEM, and the number of observations, n = 6. Different letters (a-d) indicated significant differences at $p < 0.05$.

Pentamethylenetetrazol (PTZ), an antagonist, generally induces seizures in the animal model of epilepsy (Chen *et al.* 2016) by non-competitively binding to the GABA binding sites of the $GABA_A$ receptors. Intraperitoneal (ip) injection of PTZ (80 mg/kg) induced generalised tonic-clonic seizures in mice. Hence, anti-seizure effects of Hex and Met extracts were studied in this mouse model. Mice orally administered with Hex and Met at 500 mg/kg b.w. showed significant delay (sec) of seizure appearances as 274 and 381.5 sec, respectively, compared to the control (C, 129 sec). Diazepam (Dzp, 10 mg/kg b.w.) and Mor (10 mg/kg b.w.) are agonists of the $GABA_A$ and the opioid receptors, respectively (Sigel and Steinmann 2012, Lipinski *et al.* 2019), and they also significantly delayed the appearance of seizures (Fig. 5). Hence, compounds in Hex and Met can bind with both the opioid- and the $GABA_A$-receptors to exhibit anti-seizure effects. In this experiment, Met largely prevented the induction of PTZ-induced tonic-clonic seizures in mice more than Hex, whereas Hossain *et al.* (2017) reported high analgesic activity, both from the central and the peripheral, of Hex than Met extracts.

In conclusion, acute oral administration of Hex and Met extracts of *S. apetala* fruit induced anxiolytic, hypnotic, and anti-seizure effects in mice, similar to those observed with diazepam and morphine. These effects were largely derived from the interconnected action of $GABA_A$ergic and opioidergic receptors. In the future, it is essential to formulate a dietary preparation using *S. apetala* fruit for preventing or treating anxiety, insomnia, and seizure disorders.

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