

Neuropharmacological activity of the crude ethanolic extract of *Syzygium aromaticum* flowering bud



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ABSTRACT

Background: Present study was designed to assess the possibility of *in-vivo* neuropharmacological effects of the ethanolic extract of *Syzygium aromaticum* flowering buds by using behavioral models of mice.

Methods: Anxiolytic effects of the extract were assessed using open field test (OFT), hole cross test (HCT), elevated plus maze (EPM), and hole board test (HBT) respectively; while antidepressant properties were determined using forced swimming test (FST), and tail suspension test (TST). Finally thiopental sodium (TS)-induced sleeping time test helped us to evaluate the sedative-hypnotic potential of the extract.

Results: In OFT and HCT, the movement of the mice decreased significantly ($*p < 0.005$) for the extract treated groups when compare to control. This decrease indicates the suppression of locomotor activities of mice (from 1st-5th observation periods). Moreover, the increase of the spending time in EPM open arm, and head dipping in HBT endorsed the anxiolytic-like behavior of the extract. In FST and

TST, *S. aromaticum* extract significantly ($*p < 0.05$, $**p < 0.001$) reduced the immobility time of the mice. Approx. 29% and 34% reduction of the immobility time were found in FST for 250 mg/kg, and 500 mg/kg b.w. doses respectively, which clearly indicates the presence of the antidepressant compounds in extract. Finally, TS-induced sleeping time test confirmed the potency of the sedative response of the extract (sleeping duration were 45.4±2.6 minutes for control, whereas 87.0±1.79 minutes for 500 mg/kg b.w. extract treated group). The observed neurological response may be due to binding of any phytoconstituent with gamma-amino-butyric acid (GABA_A) or benzodiazepine (BZD) receptors.

Conclusion: Our study results suggest that the ethanolic extract of *S. aromaticum* possess remarkable sedative, antidepressant and anxiolytic activities with a demand of further investigation for the drug development program.

Keywords: *Syzygium aromaticum* (SA), Open field test (OFT), Hole cross test (HCT), Elevated plus maze (EPM), Forced swimming test (FST), Thiopental sodium (TS)

INTRODUCTION

Neurological disorders are disorders of nervous system where brain, spine, and nerves are involved. Most common mental illness are- depression, anxiety, schizophrenia, autism, obsessive-compulsive disorder etc.¹ At least 20% of adult populations suffer in anxiety and depression in whole world.²⁻⁴ Heavy sweating, nervousness, dizziness, anhedonia, depressed mood, fatigue, suicidal tendencies, low self-esteem, psychomotor and gastrointestinal disturbances etc. are the symptoms of anxiety and depression.⁵ Higher cognitive centers, hippocampus, thalamus and hypothalamus of the brain and some important neurotransmitters are involved in anxiety. This is due to decrease of the inhibitory γ -amino-butyric-acid (GABA) or increase of the excitatory neurotransmission through glutamate.⁶ On the other hand, during depression the central monoaminergic function may impair.⁷

Benzodiazepines, a popular group of drug used worldwide for the patients suffer in depression and anxiety.^{8,9} However, long-term use of benzodiazepines may incur unfavorable and undesirable risks and side effects.¹⁰ As a result, it is an urgent need to search an alternative to get more efficacious, less toxic and better tolerated therapeutic agents.

S. aromaticum, a spice known as clove (family-Myrtaceae) native to Maluku islands, Indonesia.¹¹ This plant parts contain glycosides, hydroxyphenyl propens, hydrophenyl benzoic acid, hydrolyzable tannins, elagic, ferulic, salicylic acids, flavonoids, kaempferol, essential oil, α -humulen, β -pinene, farnesol, benzaldehyde, 2-heptanone, ethyl hexanoatelimonene etc. phytoconstituents.¹²⁻¹⁴ This plant is used for the stomach upset, chill, toothache, headache, cold, arthritis, rheumatism, bruises, burn, bronchitis, ulcer, asthma, minor infection, colic, nausea

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etc.¹⁵ The main phytoconstituent of *S. aromatic* is eugenol, which is reported for possessing many pharmacological properties- analgesic, anti-oxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, anti-mutagenic etc activities. Although having several uses of *S. aromaticum*, very little information is known yet about the neurological efficacy of this plant part. That's why our present study is designing to assess the neuropharmacological potency of the ethanolic extract of *S. aromaticum* flowering buds.

METHODS

Drugs and reagents

Thiopental sodium USP, and diazepam were purchased from Sigma-Aldrich corporation (USA), whereas ethanol from Merck (Germany). All other reagents used in this study were analytical grades.

Collection of plant parts

Dried flowering buds of *S. aromaticum* were collected from Riaz-Uddin Bazar, Chittagong, Bangladesh. After collection, dried bulbs were sorted from dirt, washed thoroughly by water, and then dried on sunlight. Identification of this plant part was done from Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no- 42764).

Preparation of plant extract

After sun drying for 15 days, the flowering buds were ground into coarse powder through grinding machine. Powdered materials were submerged into 2000 ml (~99.5%) ethanol in an air-tight container for 2 weeks with occasional stirring. The macerated product was allowed to filter by cloth, and then using by Whatman filter paper. After evaporating at room temperature (~25°C), we had obtained a brown sticky residue (crude ethanolic extract of *S. aromaticum* flowering buds).

Experimental animals

Both sexes (25-30g) of Swiss albino mice were collected from the animal house of Jahangirnagar University. Mice were given rodent food and water *ad-libitum* (7 days before starting the experiment to finishing the experiment). Mice were kept in metal cages [condition: (20±5)°C, RH: (55-70)%].

Animal grouping

For all experiments, mice were selected randomly and subdivided into 4 groups (each group consist of 5 mice): groups for control, standard treated, and two different concentrations of the *S. aromaticum* extract treated. After finishing each session of the experiments, mice were get off from the test apparatus, and apparatus was wiped thoroughly by using 10% ethanol.

Acute Toxicity Test

For observing acute toxicity effect, the animal were subdivided into control, and test groups respectively. *S. aromaticum* extract was administered orally at 1000, 2000, and 3000 mg/kg b.w. of mice doses respectively. Allergic symptoms, mortality of the mice were observed closely for next 72 hours period.¹⁶

Behavioral studies of anxiolytic activities

Open field test (OFT)

We have followed the method of Amin *et al.* for conducting OFT.¹⁷ The OFT board is made by white poly-wood (72x72x36 cm³). The ground of the apparatus is divided and marked into 16 equal squares; and the full apparatus is surrounded by glass wall. Mice of different groups- control (vehicle treated), standard (diazepam 1 mg/kg treated), and crude (250 mg and 500 mg/kg b.w. of mice) treated were set down in center of OFT board; and the movement of mice were recorded for 3 minutes period at 0, 30, 60, 90, and 120 minutes after administering respective doses.

Hole cross test (HCT)

HCT was done according to the procedure of Sharmin *et al.*, 2018.¹⁸ Mice were divided into control (vehicle treated), standard treated (diazepam: 1 mg/kg b.w.), and crude extract treated groups (250 mg/kg, and 500 mg/kg b.w. of mice). For this test, a wooden cage (30×20×14 cm³) was used, where there were partitions with holes. Mice were stand on one side of test chamber after dosing; and observations of their movements were done for 3 minutes periods at 0, 30, 90, and 120 minutes interval time. This test was adopted for screening anxiolytic activities of the crude extract.

Elevated plus maze test (EPM)

EPM test is useful for assessing anxiolytic-like activity of plant extract. Apparatus of EPM test was made by wood with 2 closed arms and 2 open arms crossed each other. It was placed at 40 cm above the ground and forming a plus-sign figure. Mouse was placed in middle area of the EPM device, and closely observed to its free movement. Mice were grouped randomly and given their respective doses. The spending time in each arm, and the entries in closed and opened arm were counted at 0, 30, 60, 90, 120 minutes (for 5 minutes spells) after administering respective doses. Details procedure was described in our previous paper, Rashid *et al.*, 2018.¹⁹

Hole board test (HBT)

In HBT, a wooden box (40x40x25 cm³) was placed 35 cm above the ground level. The apparatus contained 16 equidistant holes (diameter- 3 cm).

Mice of control group had received vehicle (1% tween 80 in water), standard group received diazepam (1 mg/kg b.w.) and two test groups had received extract (250 mg/kg and 500 mg/kg b.w.). Mouse was placed in center area of the box, and then allowed to move freely in board for 5 minutes. The number of head dipping during this period was recorded for individual mouse.¹⁸

Antidepressant activity test

Forced swimming test (FST)

FST is one of the popular methods for screening antidepressant-like compounds using rodent behavioral model. For this test, we followed the procedures of Amin *et al.*, 2018 with slight modification.¹⁷ This test uses cylindrical tank which was made of glass with a dimension of (30×20×15 cm³). The mice of control group were given vehicle orally, whereas diazepam (1 mg/kg b.w.) was given to the standard treated group intraperitoneally. Mice of test groups received the crude ethanolic extract of *S. aromaticum* (250 mg/kg, and 500 mg/kg b.w. of mice). Immobile time of each mouse was recorded by using a stopwatch after dosing. We have recorded the immobile time for 6 minutes period; however last 240 seconds data were taken for analysis.

Tail suspension test (TST)

TST is the rapid and reliable technique for finding antidepressant activity of the plant extract. A TST box (Dimension: 55x60x11.5 cm³) made of plastic was used for this test. Mouse was hung by its tail, and then suspended for 6 minutes period for observation. 20 mice of 4 groups were taken to be treated with standard (1 mg/kg b.w. diazepam), vehicle (control), and the plant extract (250 mg/kg and 500 mg/kg b.w.). The method described by Steru *et al.*, 1985 was followed here.²⁰ Immobile time of each mouse after hanging was counted and recorded.

Sedative-hypnotic activity test

Thiopental Sodium (TS)-induced sleeping time test

TS-induced sleeping time test was done as stated in the method of Moniruzzaman *et al.*, 2015.¹⁶ TS (40 mg/kg b.w. of mice) was injected intraperitoneally to animal after 30 minutes of the treatment of extract, standard, and vehicle (control) respectively. A stopwatch was used to record the duration for losing righting reflex, and sleep induced by TS.

Statistical analysis

Data were presented as (mean± SEM). One-way ANOVA followed by Dennett's t-test were done

using SPSS 19.0 software. Data were compared with control group (**p*<0.05, and ***p*<0.001 were significant and highly significant values). Origin Pro (Ver.-8.5, Origin Lab. Corp., USA), and Microsoft office excel (2010) are used for preparing graphs.

RESULTS

In this study, the neuropharmacological activities of crude ethanolic extract of *S. aromaticum* flowering bud were assessed. We found that crude extract at 1000, 2000, and 3000 mg/kg doses didn't produce any adverse or allergic symptoms during 72 hours observation period. As a result it can be said that the studied crude extract may be safe upto 3000 mg/kg b.w. of mice. Based on this cytotoxic data, we have selected the doses (250 and 500 mg/kg b.w. doses) which were much lower than toxic concentration (>3000 mg/kg b.w. of mice).¹⁶

Evaluation of the behavioral studies

Open Field Test

In OFT, *S. aromaticum* extract at both doses (250, and 500 mg/kg b.w.) significantly (**p*<0.05, and ***p*<0.001) reduce the movement of mice with dose dependant manner (Figure 1). For 500 mg/kg b.w. of mice dose, the total number of squares traveled reduced significantly upto 120 minutes.

Hole Cross Test

The experimental result of HCT is shown in (Figure 2). Mice treated with *S. aromaticum* extract (250, and 500 mg/kg b.w.) decrease locomotion activities significantly (**p*<0.05, ***p*<0.001). The subsequence of this reduction of the movement was found from 2nd (30 minutes) to 5th observation (120 minutes) after treatment. We found the dose dependant response of *S. aromaticum*, which is comparable to the decrease of the movement by standard drug (diazepam: 1 mg/kg b.w.)

Elevated plus maze test

EPM test of *S. aromaticum* ethanolic extract is used to observe the anxiolytic-like effect as reported previously. Treatment of mice by the plant extract (500 mg/kg) showed that the time duration spent in open arm of EPM increased significantly (**p*<0.05, ***p*<0.001); whereas in closed arm the time decreases slightly (Figure. 3). Plant extract at the conc. (250 mg/kg) also shows similar activity, although the activity is not significant. We found dose dependant response of the extract. The higher duration of residing in open arm mean the lower level of anxiety of mice.

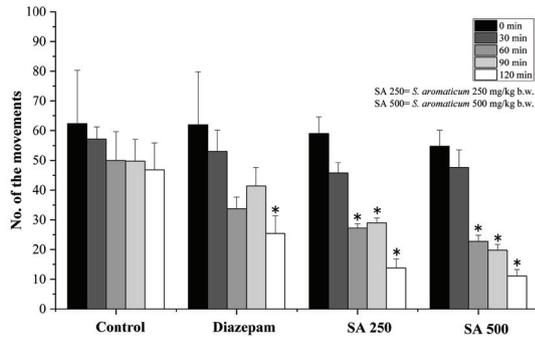


Figure 1 Effect of the ethanolic extract of *S. aromaticum* flowering bud in open field test of mice.

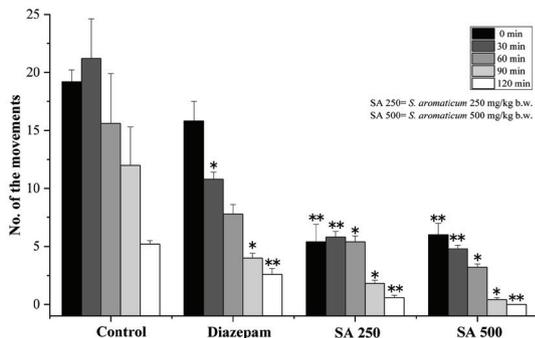


Figure 2 Effect of the ethanolic extract of *S. aromaticum* flowering bud in hole cross test of mice.

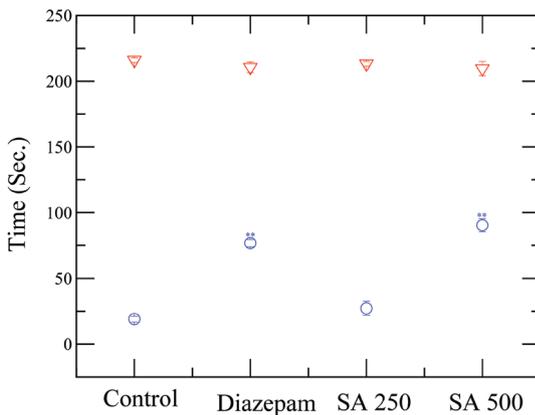


Figure 3 Effect of *S. aromaticum* extract in elevated plus maze test of mice. Open triangle (∇), and open circle (\circ) represent the time spent in close arm and open arm of the EPM apparatus respectively. SA 250 = *S. aromaticum* 250 mg/kg b.w., and SA 500 = *S. aromaticum* 500 mg/kg b.w. of mice.

Hole Board Test

In HBT, the ethanolic extract of *S. aromaticum* at 500 mg/kg b.w dose significantly increased the number of head dipping. Approx. (46.20 ± 3.79), (75.00 ± 4.55) head dips were found for

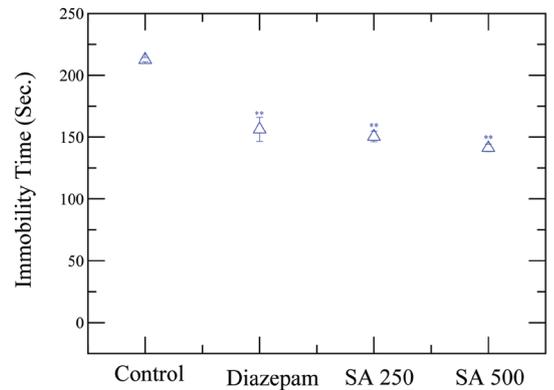


Figure 4 Effect of *S. aromaticum* ethanolic extract on forced swimming test of mice. Open triangle (\triangle) represents the immobility time of the mice. SA 250 = *S. aromaticum* 250 mg/kg b.w., and SA 500 = *S. aromaticum* 500 mg/kg b.w. of mice.

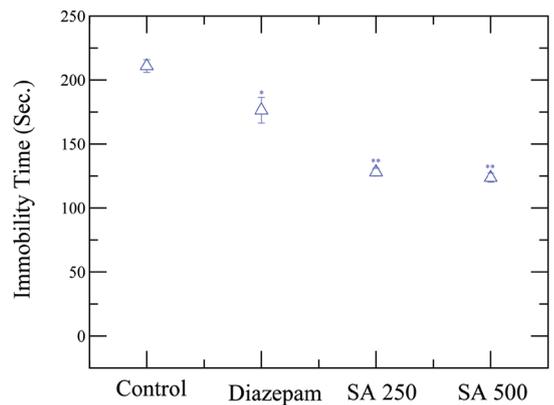


Figure 5 Effect of *S. aromaticum* ethanolic extract on tail suspension test in mice. Open triangle (\triangle) represents the immobility time of the mice. SA 250 = *S. aromaticum* 250 mg/kg b.w., and SA 500 = *S. aromaticum* 500 mg/kg b.w. of mice.

250, and 500 mg/kg b.w. extract respectively. The result is comparable to the standard drug diazepam (59.40 ± 7.61). The overall result is shown in Table 1.

Evaluation of the antidepressant activity test

Forced Swimming Test

Administration of both doses of *S. aromaticum* ethanolic extract significantly ($*p < 0.05$, $**p < 0.001$) decreases the immobility time in FST. The effect follows dose dependant trend which is plotted in (Figure 4).

Tail Suspension Test

The result of TST test is shown in Figure 5 It was found that ethanolic extract of *S. aromaticum*

Table 1 Effect of *S. aromaticum* extract in hole board test of mice.

| Group | Dose | Number of head dipping |
|----------------------|-------------------------------|------------------------|
| Control | 0.1 ml/mouse (oral) | 27.80 ± 4.97 |
| Diazepam | 1 mg/kg b.w. of mice (i.p.) | 59.40 ± 7.61* |
| <i>S. aromaticum</i> | 250 mg/kg b.w. of mice (oral) | 46.20 ± 3.79 |
| <i>S. aromaticum</i> | 500 mg/kg b.w. of mice (oral) | 75.00 ± 4.55** |

Values are presented as mean ± SEM (n=5). One way ANOVA followed by Dunnet's t-test were done. * $p < 0.05$, ** $p < 0.001$ as compared to control.

Table 2 Effect of *S. aromaticum* ethanolic extract on thiopental sodium-induced sleeping time test of mice

| Group | Dose | Onset of sleeping (minutes) | Sleeping duration (minutes) | Effect (%) |
|----------------------|-------------------------------|-----------------------------|-----------------------------|------------|
| Control | 0.1 ml/mouse (oral) | 34.6 ± 0.93 | 45.40 ± 2.60 | 0 |
| Diazepam | 1 mg/kg (i.p.) | 18.4 ± 0.93** | 51.40 ± 6.40 | 13.21 |
| <i>S. aromaticum</i> | 250 mg/kg b.w. of mice (oral) | 19.8 ± 0.80** | 74.00 ± 2.92** | 62.99 |
| <i>S. aromaticum</i> | 500 mg/kg b.w. of mice (oral) | 13.0 ± 0.77** | 87.00 ± 1.79** | 91.63 |

Values are presented as mean ± SEM (n=5). One way ANOVA followed by Dunnet's t-test were done. * $p < 0.05$, ** $p < 0.001$ as compared to control.

decreases immobility time of the mice proportionally with increase of extract concentration. For 250, and 500 mg/kg b.w. of the extract, immobilization times were (128 ± 2.98) and (123.6 ± 3.56) seconds respectively. On the other hand, immobilize time was (176.4 ± 10.12) seconds for diazepam. The result is significant (* $p < 0.05$, ** $p < 0.001$), and follows dose dependant response.

Sedative-hypnotic activity test Thiopental Sodium (TS)-induced sleeping time test

It was found that *S. aromaticum* (250, and 500 mg/kg b.w.) significantly shorten the onset of sleeping time, and also increase the duration of sleep. The overall result has been shown in Table 2. We found that the effects of *S. aromaticum* extract follow dose dependant manner. Maximum sleeping duration (87 ± 1.79) minutes was found for 500 mg/kg b.w. of extract. In contrast, this value is (51.4 ± 6.4) minutes diazepam (1 mg/kg b.w.).

DISCUSSION

Plants are considered as alternative sources of the therapeutic agents from the early age of human civilization. In modern era, over 50% of the drugs come from natural origins (plant and animal sources). These natural products are playing important roles in drug development program.²¹ On the other hand, proper diagnosis and treatment of the patients suffer in anxiety and depression is much

challenging, moreover not so satisfactory level still now.²² Although many new drugs developed in last two decades, none of them is perfect choice as the side effect is not in acceptable limit. That's why we try to find out more efficient, safer and cost effective neurological agents from plant origin. In this study, we try to assess the neuropharmacological efficacy of *S. aromaticum* ethanolic extract using swiss albino mice model.

OFT and HCT are the two reliable neuropharmacological models used to assess the anxiolytic-like efficacy of the compounds. In OFT study, we found that the extract treated groups at both doses (250, 500 mg/kg b.w.) significantly (* $p < 0.05$, ** $p < 0.001$) decreased the number of movement of mice when compared to the vehicle treated group (control). Highest decrease was found after 120 minutes of the extract administration. We found dose dependant response of the extract, and the efficacy is comparable to the effect of standard drug (diazepam). Anxiolytic agents can decrease the fearful attitude of the mice in OFT and HCT apparatuses which can decrease the locomotor activity of mice.^{23,24} In both tests, motor activities of the mice decreases gradually from 1st to 5th observation periods (as movement of mice decreases), which confirm the time dependency of the anxiolytic effect. The anxiolytic-like potency of *S. aromaticum* was also assessed by EPM and HBT tests. EPM is one of the reliable and highly sensitive test methods for identifying selective anxiogenic and anxiolytic effect of the agents. The preference of spending maximum time in open arm of the EPM apparatus is the reflection of anxiolytic-like effect.¹⁸ We found that 500 mg/kg b.w. of *S. aromaticum* extract (given to the mice orally) can increase the spending time in opened arms significantly rather than closed arms. In HBT, the increased number of head dipping reflects an indication of the anxiolytic-like activity.²⁵ In our study, we found that 500 mg/kg of *S. aromaticum* ethanolic extract significantly increased the number of head dipping (almost 3 times than control group), which endorsed the anxiolytic-like efficiency of the extract.²⁶ Phytochemical screening claimed that *S. aromaticum* possesses high amount of essential oil- eugenol (major constituent), eugenyl acetate, β -caryophyllene etc.²⁷ Nassar *et al.*, 2007 isolated some other phytoconstituents from the ethanolic fraction of *S. aromaticum*, like-flavonoids (tamarixetin 3-O-b-D-glucopyranoside, ombuin 3-O-b-D-glucopyranoside, quercetin), aldehyde (ferulic aldehyde), limonin, phenol, hydrolizable tannins, elagic, salicylic acids etc.²⁸ Presence of these phytoconstituents may be responsible for the CNS effects.²⁹ The possible reason of the anxiolytic effect is due to hyperpolarization of CNS by the interaction with gamma-amino-butyric

acid (GABA_A) or benzodiazepine (BZD) receptor. GABA is a well-known inhibitory neurotransmitter in CNS, and the anxiolytic activities of most of the drugs show by acting on GABA_A receptor. Therefore our proposed hypothesis stands that the anxiolytic-like effect of *S. aromaticum* extract may be due to interacting/binding of any phytoconstituent with GABA_A receptor.^{29,30}

Anxiety and anxiety disorders are found in acute phase of depression. Antidepressant drugs help to sleep better, and also used for treating social anxiety disorder, generalized anxiety disorder etc. That's why strong antidepressant drugs with anxiolytic activity are very important. For screening the antidepressant activity of the agents, TST and FST are valid, fast and reliable methods.³¹ Duration of immobility in TST and FST is considered as screening parameter of this activity. Both doses of *S. aromaticum* extract significantly decreased the period of immobility of mice (approx. 29% and 34% reduction of immobility time than control were found for 250, and 500 mg/kg b.w. doses). The effect follows dose dependant manner and also comparable with standard drug diazepam. *S. aromaticum* plant extract contains many constituents including flavonoids, tannin, glycosides etc. Flavonoid and tannin possesses antioxidant activities. Thus they can prevent oxidative stress which is believed one of the reasons of depression. Again they also can ward receptors and enzyme activities.³² In intestine, flavonoid and glycoside can be hydrolyzed to form aglycon; and after absorption they convert into conjugated metabolites. These metabolites transport to the brain tissues and exert antidepressant activity.³³

Thiopental Sodium (TS)-induced sleeping time test was performed for assessing sedative-hypnotic potential of *S. aromaticum* ethanolic extract. TS is a hypnosis-inducing agent, and it induces hypnosis by increasing GABA mediated inhibition in post synaptic membrane via allosteric modification of GABA_A receptors. *S. aromaticum* extract give significant dose dependent ($*p < 0.05$, $**p < 0.001$) sedation. The onset of sleeping for 500 mg/kg b.w. dose gives much quicker response than diazepam (1 mg/kg b.w). The sedative effect of this extract may be due to the hyperpolarization by interacting with GABA_A receptor which may increase the GABA concentration in brain.¹⁸ So it is assumed that the effect of *S. aromaticum* extract may be similar as benzodiazepines.

CONCLUSION

Based on the results found in this study, we have concluded that crude ethanolic extract of *S. aromaticum* possesses significant antidepressant, sedative,

and anxiolytic-like efficacies. The potency of the extract to increase the sleeping duration in sleeping time test, decreasing the immobility time in FST and TST, decrease of locomotor activities in OFT and HCT, increase of the head dipping in HBT- all the tests endorsed the significant neurological activities of *S. aromaticum* extract. Therefore, there is a good possibility to have bioactive ingredients in this extract for the treatment of insomnia, depression and anxiety. However for confirming the neurological activity and potential therapeutic use, further research is suggested specially the isolation of the responsible bioactive compounds and their clinical trial are recommended.

LIST OF ABBREVIATIONS

S. aromaticum: *Syzygium aromaticum*; OFT: Open Field Test; HCT: Hole Cross Test; EPZ: Elevated Plus Maze; HBT: Hole Board Test; FST: Forced Swimming Test; TST: Tail Suspension Test; TS: Thiopental Sodium; GABA: Gamma Amino Butyric Acid.

DECLARATIONS

Ethics approval and consent to participate

Experiments in this study have been done according to the laws of institutional ethical committee. 'Principles of the laboratory animal care' (NIH publication no. 85-23, revised 1985) and 'national animal care laws' were maintained strictly during conducting the experiments.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

All raw data and analyzed data found in this study have been included in the manuscript.

COMPETING INTERESTS

Authors have none to declare.

FUNDING

Not applicable.

AUTHOR'S CONTRIBUTIONS

Designing of the study was done by MMH and MMOR. Manuscript was written by MMOR and

TDA, MMH and AHMMU actively participated in the experiments. MSR helped to improve writing quality, and revised manuscript in current version. NR helped us to identify the plant part from Bangladesh National Herbarium. All authors approved the final version of this manuscript.

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