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Anxiolytic activity of methanolic extract of *Costus speciosus* flower on swiss albino mice

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Abstract
Costus speciosus is a flowering plant of spiral ginger family. The present study was carried out to evaluate the anxiolytic activity of *Costus speciosus* flower. The anxiolytic activity was studied using two methods (Elevated plus maze and Hole board test). The methanolic extracts were administered to swiss albino mice orally at doses of 200 and 400 mg/kg. The extract at dose of 400 mg/kg increased the time spent in the open arm of Elevated plus maze and the number of head dipping in hole board was increased at doses of 200 and 400 mg/kg. The results reveal the presence of anxiolytic activity of *Costus speciosus* flower.

Keywords: Anxiolytic activity, *Costus speciosus* flower, elevated plus maze, hole board

Introduction
Anxiety disorders refer to a group of mental disorders characterized by feelings of anxiety and fear, including generalised anxiety disorder (GAD), panic disorder, phobias, social anxiety disorder, obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) [1]. Affecting between 15-20% of youth, anxiety disorders are among the most prevalent psychiatric conditions in children and adolescents [2]. Anxiety disorders are more common among females than males [3]. Current FDA (Food and Drug Administration) approved treatments for anxiety disorder includes selective serotonin reuptake inhibitor, serotonin norepinephrine reuptake inhibitor, benzodiazepine, tricyclic antidepressants, monoamine oxidase inhibitor, antihistamine and others [4]. Modern pharmacological treatments for anxiety disorders are safer and more tolerable than they were 30 years back. Regrettably, treatment efficacy and duration of anxiolytic drug have not been improved in most cases yet. Therefore, identification of better anxiolytic drug becomes very important [5, 6, 7]. However, many plants have been found to have antianxiety effects at satisfactory level [8, 9].
Costus speciosus also known as crepe ginger is cultivated in Bangladesh as vegetable and medicinal plant. Phytochemical screening of *C. speciosus* detected the presence of alkaloids, glycosides, steroids, phenolic, flavonoids, polyphenols, tannins, and β-carotene. Besides, diosgenin, β-sitosterol, furostanol saponins-costusosides, β-D-glucoside, prosapogenins, dioscin, gracillin, dihydrophytyl plastoquinone, and α-tocopherolquinone were also isolated from *C. speciosus* [10, 11]. The plant has been found to possess many pharmacological activities such as antibacterial, antifungal, anticholineesterase, and antioxidant, antihyperglycemic, antiinflammatory, analgesic, antipyretic, antidiuretic, larvicidal, antistress and estrogenic activity [12]. According to literature survey, research has been conducted on anxiolytic effect of *C. speciosus* rhizome (modified plant stem that sends out roots and shoots from the nodes of this plant) and found to possess significant activities [13] but the anxiolytic activity of *C. speciosus* flower has not been reported yet. Therefore, our aim was to investigate the anxiolytic effect of *C. speciosus* flower extracts on swiss albino mice using Elevated plus maze and hole board method.

Materials and methods
Collection of flower and Extraction
The flowers were collected from Maheshkhali, Bangladesh. This plant was identified and authenticated by National Herberium, Bangladesh. The flowers were washed well and dried. The dried flowers were then ground into a coarse powder with the help of a suitable grinder. The powered material was soaked in methanol for 15 days with occasional shaking and stirring. The whole mixture was first filtered using white cotton cloth followed by filttration through whatman filter paper. The obtained methanolic extract was evaporated using the traditional spontaneous natural vaporization method at room temperature by covering with aluminium foil with small pores to facilitate evaporation of methanol.

Animals

Swiss-albino mice of male sex, aged 4-5weeks and weight between 20-30gm, obtained from the animal house of Jahangirnagar University. They were kept in standard environmental condition and fed ICDDRB formulated rodent food and water (ad-libitum). As these animals are very sensitive to environmental changes, they are kept before the test for at least 3-4 days in the environment where the experiment will take place. Swiss albino mice were divided into four groups of five animals each and given the respective treatment. The control group (1st group) was treated with distilled water, the standard group (2nd group) was treated with diazepam 1mg/kg intraperitoneally, and the 3rd group and 4th group were respectively treated with the methanolic extracts of *C. Speciosus* at doses of 200 and 300 mg/kg orally.

Elevated plus maze test

This test was carried out according to the method described by lister [14]. The Elevated plus maze was consisted of two open arms (35 x 5 cm) crossed with two closed arms (35 x 5 x 15 cm). The arms were connected together with a central square of 5 x 5 cm. The apparatus was elevated to the height of 40 cm above the floor. The mice were given their respective treatment. After one hour of treatment, mice were individually placed in center square facing either one of the open arms. The time spent in both the open and closed arms was recorded for 5 minutes. An entry was defined as having all four paws within the arm [15].

Hole board test

Hole board test is an experimental model used for the screening of anxiolytic activity based on number of head dips in holes [16,17].The hole-board apparatus was consisted of a wooden box (40 x 40 x 25 cm) with 16 holes (each of diameter 3 cm) evenly distributed on the floor. The apparatus was elevated to the height of 35 cm. The mice were given their respective treatment. One hour after treatment, each mouse was placed in turn at one corner of the board with the animal subsequently moving about and dipping its head into the holes. The number of head dips during a 5 minutes period was recorded for individual mouse.

Statistical analysis

Statistical analysis was done by one way ANOVA (analysis of variance) followed by dunnetts' test compared test with control group at $*p<0.05$, $**p<0.001$

Results

Elevated plus maze test

In this test, *C. speciosus* at the dose of 400mg/kg and diazepam 1mg/kg produced significant increase in time spent in open arm. The *C. speciosus* (400mg) was found to be statistically significant at $p<0.05$. The result for anxiolytic activity of the extract is shown in Table 1 in case of Elevated plus maze method.

Table 1: Effect of *C. speciosus* flower extract on spending time in open arm

Group	Treatment	Time spent in Open arm in sec	Time spent in Closed arm in sec
Control (1st group)	Placebo(10ml/kg)	10.8 ± 1.24	289.2 ± 1.24
Standard (2nd group)	Diazepam(1mg/kg)	66 ± 2.8**	234 ± 2.51
Test (3rd group)	*C. speciosus* (200mg/kg)	13.2± 1.46	286.8 ± 1.46
Test (4th group)	*C. speciosus* (400mg/kg)	21.4 ± 2.29*	278.6 ± 2.29

Each values is represented as mean ± SEM (n = 5).

Hole board Test

In this study, diazepam and all doses of extracts significantly increased the number of head dipping on hole board. The *C. speciosus* flower extract (200mg/kg) found to be statistically significant at $p<0.05$ and at a dose of 400 mg/kg was found to be significant ($p<0.001$). The result for anxiolytic activity of the extract is shown in Table 2 in case of Hole board method.

Table 2: Effect of *C. speciosus* flower extract on number of head dipping

Group	Treatment	Dose (mg/kg)	Number of Head Dipping					Mean ± SEM
			M1	M2	M3	M4	M5	
Control (1st group)	Placebo	10ml/kg	8	7	6	12	15	9.6 ± 1.69
Standard (2nd group)	Diazepam	1mg/kg	40	38	44	29	30	36.2 ± 2.9**
Test (3rd group)	*C. speciosus*	200mg/kg	18	13	20	18	28	19.44±2.44*
Test (4th group)	*C. speciosus*	400mg/kg	22	31	26	38	24	28.2 ± 2.87**

Discussion

Anxiety may be caused due to the involvement of neurotransmitters and transporters of noradrenergic, serotonergic, glutaminergic, and GABAergic neurons and hormones (neuropeptide Y, CCK) [18,19]. Decreased level of GABA (gamma-aminobutyric acid) in CNS (Central Nervous System) plays a vital role in causing anxiety [20]. In addition to the GABA release, 5‑HT also plays a major role in the development of anxiety [21]. In our study, the anxiolytic effect of *C. speciosus* have been studied by the two experimental models of anxiety (Elevated plus maze and Hole board). In the Elevated plus maze, animals find the open arms to be unprotective and more anxiogenic than the closed arms. Animal hence prefers to spend more time and shows normal rearing behavior in the closed arm. Anxiolytic drugs increase the number of entries, time spent, and rearing in the open arms [22, 23]. In our experiment, the methanolic extract at dose of 400mg/kg significantly increased the time spent in open arms which indicates the presence of anxiolytic activity in the studied plant flower.

The Hole board test is useful for modelling anxiety in animals. Using this test, an anxiolytic state can be explained by an increase in head dipping behaviors [24]. The *C. speciosus* at doses of 200mg/kg and 400mg/kg significantly increased the number of head dipping which again confirms the presence of anxiolytic activity of *C. speciosus* flower extract under study.

Conclusion

Discovery of drug using natural products is challenging but a promising task. The flower of *C. speciosus* bears anxiolytic activity; hence this flower can be a good source of natural anxiolytic drug. Our pharmacological studies scientifically prove the anxiolytic effect of *C. speciosus* flower by increasing the time spent in open arm and the number of head dipping in Elevated plus maze and Hole board test respectively.

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