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ANTIDEPRESSANT AND ANXIOLYTIC EFFECT OF ETHANOL EXTRACT OF FICUS PLATIPHYLLA STEM BARK IN MICE

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ABSTRACT

The present study evaluated the effect of ethanol extract of Ficus platiphylla stem barks in the doses of 20, 40 and 80 mg/kg on anxiety and depression using mice as a model. Ethanol extract of Ficus platiphylla stem barks increased significantly the number of square crossed (center and peripheral), duration at the center of the field and rearing frequency, while there was significant reduction in duration of grooming in open field test. In the elevated plus maze experiment, the number of entries and duration of time in the open arms increased significantly with Ficus platiphylla stem barks extract treated groups. These aforementioned behavioural indices were also completely nullified by flumazenil, a benzodiazepine receptors antagonist and pindolol a β-adrenoceptors blocker or 5-HT 1A/1B receptor antagonist. In the light and dark experiment, the time spent as well as the frequency in the light compartment and the number of the light-dark transitions increased significantly with Ficus platiphylla stem barks extract treatment. The extract was able to significantly reduced the immobility time, while activity of the mice in swimming and climbing increases significantly. In conclusion, the present study revealed the anxiolytic and antidepressant effects of the ethanol extract of Ficus platiphylla stem bark and the propable mechanism of action might be mediated via benzodiazepinergic and noradrenergic pathway.

INTRODUCTION

Anxiety is one of the most common mental disorders that influence all ages, the young and the old groups of the society.[1] This disorders are classified under the following sub-heading; Panic attach, Social phobia, Agoraphobial and Specific Phobial.[2] It has become one of the research area of interest among the psychopharmacologist. The following are the major classes of drug that can be found over the counter for the management of anxiety disorder: selective serotonin-reuptake inhibitors (SSRIs) and benzodiazepines.[3]

However, most of these drugs have been reported with various adverse effects such as impaired coordination, trembling, grogginess, muscle relaxation, hepatotoxicity and dizziness.[4] These aforementioned adverse effect prompted the scientist in search for new anxiolytic agents that may have a fast onset of action with fewer side effects and a wider safety margin. Medicinal plants have been reported to be a good source to find alternative remedies for these disorders.

Ficus platyphylla belong to the family Moracea, it is commonly called gutta percha tree. The plant is found in the savannah areas. It is a very common tree in the northern part of Nigeria and commonly called 'gamji' among the Hausas and 'Obobo' in Yoruba language speaking of Southwest. Part of this plant like: seeds, bark and leaves have been grounded together or singly and found its used ethnomedicinally in enhancing fertility and treatment of epilepsy, psychosis, depression, pain and inflammation.[5] Many Scientist have recently studied the plant to validate the traditional claimed and it has been shown to have analgesic, anti-inflammatory and CNS effects.[6][7][8] The plant was also reported to be save when used in long term basis as previously described.[9] The plant has been scientifically proven to promotes fertility in female Rattus norvegicus Wistar strain rats.[10] We therefore decided to investigate the neurobehavioural effect of the stem bark extract of F. platiphylla using mice.

MATERIALS AND METHODS

Plant material

Collection and identification of plant materials

Fresh stem bark of Ficus platyphylla was collected from Owode, Ede Osun State, South West, Nigeria. The plant was identified by Mr. Bernard Omomoh of the herbarium unit, Department of Botany, Obafemi Awolowo University Ile-Ife, where voucher specimen was deposited (voucher number 17235).

Preparation and extraction of plants material

Ficus platyphylla stem bark weighing 1 kg was air-dried for eight weeks and reduced to coarse powder form using electric blending machine. Air-dried powder of Ficus

platyphylla was macerated in 3 litres of absolute ethanol and placed on a mechanical shaker for 24 hours to extract. It was then filtered and concentrated to dryness using rotary evaporator (Buchi Rota Vapour R110) followed by freeze drying. The crude ethanol extract (CEE) obtained was preserved in the freezer until ready for use.

Animal Materials

The animals used in the study were mice (Swiss strain, male, 20-30 g). They were obtained and housed in Animal House, College of Health Sciences Osogbo, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso.

They were kept in standard cages with a maximum of six animals in a cage. The animals were housed under standard environmental conditions in the Department of Pharmacology and Therapeutics, of the University. Animals were fed with standard diet (Ladokun feeds Ltd, Ibadan) for two weeks prior to experimentation and allowed free access to clean drinking water.

Behavioral Evaluation

Open Field Activity Test (OFT)

The open field apparatus was made of white polywood and the dimension was 72 × 72cm with 36cm walls. The floor was marked with Red lines and which made clear through the Plexiglas floor. The mice were grouped into five randomly with five animals per group. Group 1, received the vehicle (normal saline, 10 ml/kg), group 2 received diazepam (4 mg/kg) as standard while groups (3-5) received the extract in the doses of 20, 40, and 80 mg/kg body weight. They were all administered intraperitoneally 30min before the test.

Each mouse was placed in the open field box, allowed to explore the environment for 6 min and their behaviors were recorded. The time spent at the center square, total number of the lines crossed, frequency of rearing (i.e number of times the animal stood on its hind limb in air or placed it hind limb on the wall of the box), and grooming behavior (i. e period the animal spent licking or scratching its body).[11]

Elevated Plus-Maze Test (EPM)

This experiment is used to evaluate exploration, motor behavior and to determine anxiety as well. Similarly, the mice were randomly grouped into five with five animals per group.

Group 1, received the vehicle (normal saline, 10 ml/kg), group 2 received diazepam (4 mg/kg) as standard while groups (3-5) received the extract in the doses of 20, 40, and 80 mg/kg body weight. They were all administered intraperitoneally 30min before the test. The method used by Foyet et al. [12] was used in evaluating the possible anxiolytic effects of Ficus platiphylla.

The EPM is made of four arms in the dimension of 40cm long and 10cm wide, the two arms were made to oppose each other. The maze was raised by 45cm above the ground. Two arms were enclosed by walls of 25cm high while, the other two arms were exposed. Thirty minutes after the intraperitoneal injection of the extract or saline or diazepam, each mouse was placed at the middle of the maze facing one of the enclosed arms.

Observation was made of the mouse for a period of 5min test, the number of time each mouse entered open and enclosed arms and the time spent in open and enclosed arms, were recorded as described in the previous study [12, 13]. Entry of each mouse into an arm was defined when all four paws were placed into the arm. After each test, the maze was thoroughly cleaned with 70% ethanol solution and allowed to dry before the next animal.

In order to access the mechanism involved, mice were subjected to the co-administration of the aqueous extract of Ficus platiphylla stem bark and pindolol (10 mg/kg) or flumazenil (10 mg/kg). The extract in the doses of 20, 40, and 80 mg/kg body weight was administered intraperitoneally thirty minutes after the mice have been pre-treated with flumazenil and or pindolol prior the test

Light-Dark Transition Test (LDB)

The LDB test was performed as described by Gong et al. [14, 15], with minor modifications.

The box was made of polywood with the dimension of 45 × 27 × 27cm and has two chambers with an opening (6 × 6cm) located at the floor level in the center of the middle wall. Division of the floor was made into 9 × 9cm squares and was covered with Plexiglas. We have two chambers, the small (black) and the big chambers (white) with the dimension of 18 × 27cm and 27 × 27cm respectively.

The lamp 60 W located 40cm above the middle of the white chamber to illuminate it. The mice were randomly grouped into five with five animals per group. Group 1, received the vehicle (normal saline, 10 ml/kg), group 2 received diazepam (4 mg/kg) as standard while groups (3-5) received the extract in the doses of 20, 40, and 80 mg/kg body weight. They were all administered intraperitoneally 30min before the test.

During the test, each mouse was placed at the middle of the light compartment with their back to the dark compartment, and then observation was made for transition behaviour over a period of 10min, this including the latency time (i.e latency before entering the dark compartment), the transition number, and the total time each mouse spent in the light compartment.[16,17]

The box was then cleaned with 70% ethanol at the end of each experiment.

Forced Swimming Test (FST)

The Force swimming test is an experimental model used for assessing antidepressant activity [18]. The immobility observed after the animal was placed in an inescapable box or cylinder containing water is an indication of cessation of persistent escape-directed behavior according to [19]. The antidepressant effects of the ethanol extract of F. platiphylla stem bark was evaluated, using the method described by Foyet et al.[12] The mice were grouped into five randomly with five animals per group. Group 1, received the vehicle (normal saline, 10 ml/kg), group 2 received fluoxetine (10mg/kg, i.p) as standard while groups (3-5) received the extract in the doses of 20, 40, and 80 mg/kg body weight.

They were all administered intraperitoneally 30min before the test. The mouse was placed into transparent Plexiglas cylinder with the dimension of 50cm high and 20cm wide filled to a 30cm depth with water at room temperature. The mouse was observed to swim for 15min before being removed and returned to their cages.

The pretreated mice with extract or vehicle or fluoxetine were also observed to swim as explained above. During the period of the experiment, the following behavioral indices were recorded: duration of floating with little movements while the head is above water or immobility time, time spent with active swimming movements, and climbing time (time spent when the animal is trying to escape).

When the climbing or swimming responses increases and a reduction in immobility time in animal is observed, this were considered as behavioral profiles consistent with an antidepressant-like action.[18]

Statistical Analysis

Data were analyzed using One-way analysis of variance (ANOVA) followed by post-hoc tests (Student Newman Keul's) which was used to determine the source of a significant effect. Results were expressed as Mean ± SEM., while $p<0.05$ was taken as accepted level of significant difference from control or vehicle.

RESULTS

Effects of the Extract in the OFT

The extract significantly increases rearing time in a dose-related manner (Fig 1a). In contrast, number of lines crossed as well as frequency of rearing in diazepam treated group decreased significantly (Fig 1c). The grooming was significantly reduced by the extract and the standard drug, when compared with the control treated group (1b).

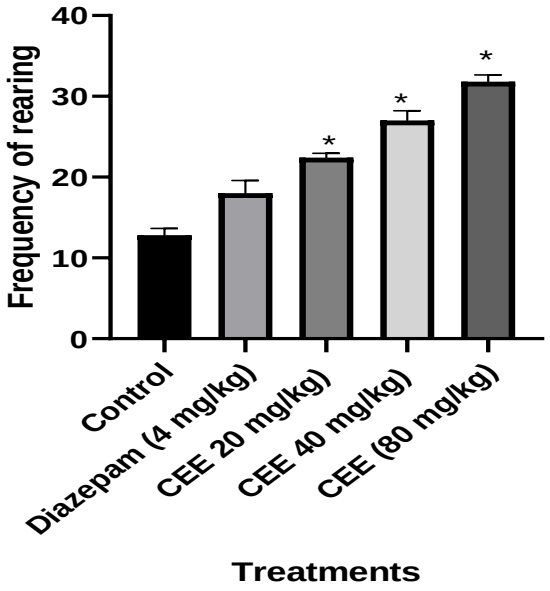

Figure 1a: Effect of extract on frequency of rearing. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

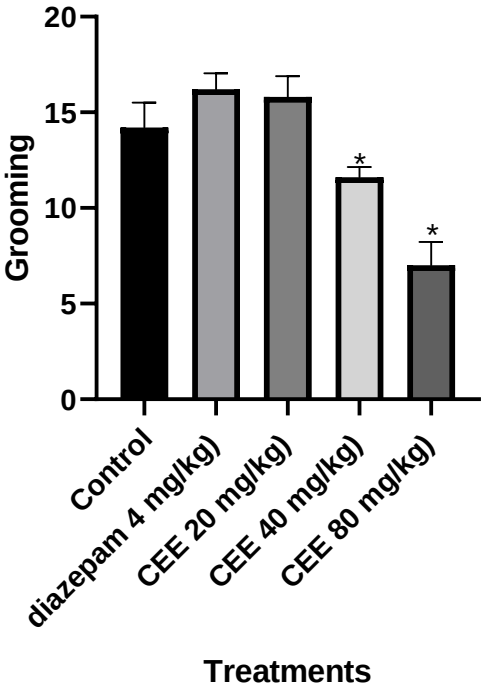

Figure 1b: Effect of extract on grooming behavior. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

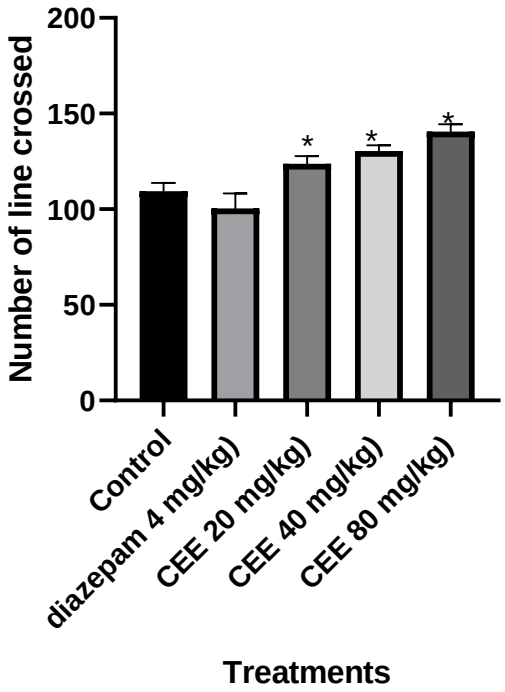

Figure 1c: Effect of extract on line crossed by the mice in open field.
Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

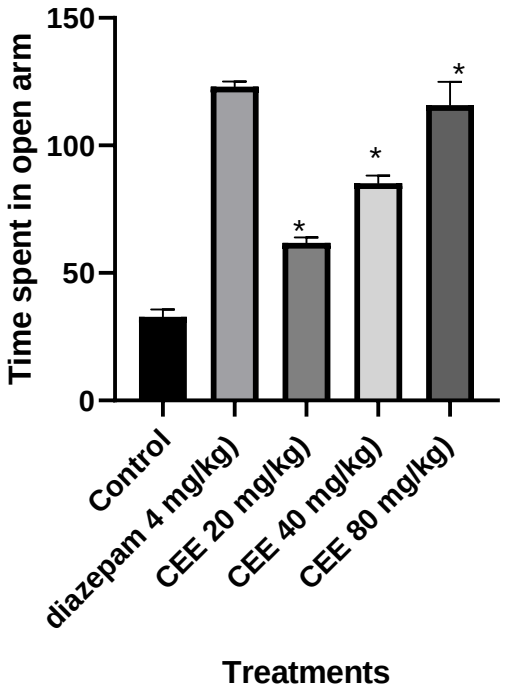

Figure 2a: Effect of extract on the time spent in open arm entries.
Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

4.2. Effects of the Extract in the EPM

The time spent by the mice in opened arms was significantly increased compared with the control group (Figure 2a). Figure 2(b) shows that the number of entries in the open arms was significantly increased in animals treated with the extract and the standard drug. However, duration and the numbers of entries in the enclosed arms were significantly reduced in the extract treated groups compared to control animals.

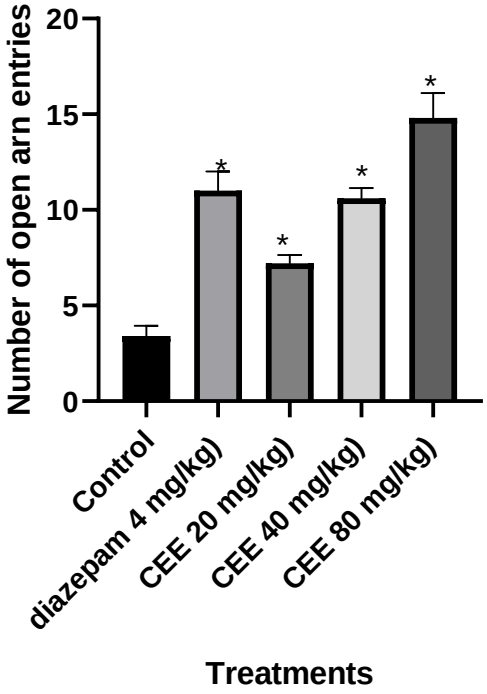

Figure 2b: Effect of extract on the number of open arm entries.
Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

4.3. Blocking effect of Pindolol and Flumazenil on the Anxiolytic Effect of the Extract

The anxiolytics behavioral effect of the ethanol extract of F. platiphylla stem bark was completely blocked by pindolol and flumazenil significantly. While, pindolol at the same dose failed to abolish the anxiolytic-like effect of diazepam, flumazenil blocked or reduced the open arms entries of mice (Fig. 3).

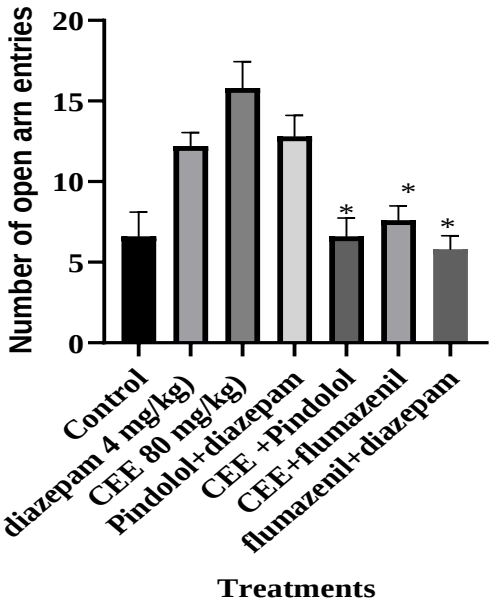

Figure 3: Blocking effect of flumazenil and pindolol on the anxiolytic effect of CEE.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

4.4. Effects of the Extract in the LDB

The latency time, number of transitions, and the time spent in the light compartment increased significantly ($p<0.5$) at the maximum dose of the extract treated group. The number of transitions and the time spent in the light compartment significantly ($p<0.5$) increased while, the latency time decreased in diazepam treated group (Table 1).

Table 1: Effects of crude ethanol extract of F. platiphylla stem bark on the light-dark transition test with mice.

Treatments	doses (mg/kg)	Time spent in lighted box (sec)	Time spent in dark box (sec)	No. of crossings
Vehicle	0	112.5±7.23	185.8±11.21	15.21±3.42
Diazepam	1	187.7±10.51	121.1±3.23	23.50±1.76
CEE	20	196.3±7.96	132.7±8.73	27.71±1.97
CEE	40	231.5±6.78	141.3±4.65	29.1±1.33
CEE	80	257.6±3.26	142.8±3.71	32.7±5.61

**Values are recorded as means±SEM (n=5).

*Values are statistically significant ($p<0.05$) in relation to control. One-way ANOVA follow by Newman-Keuls Multiple Comparison tests.

Effects of the Extract in the FST

The mice treated with the extract reduced the immobility time significantly. This activity was also observed in animals treated with fluoxetine at 10mg/kg (Table 2). Conversely, the swimming or the climbing time (Figure 4) was significantly increased by the ethanol extract of F. platiphylla stem bark.

Conversely, Fluoxetine did not significantly have effect on climbing time, but did increase swimming time.

Table 2: Effect of ethanolic extract of *F. platiphylla* on forced-swimming test.

Treatments	Doses (mg/kg)	Immobility time (second)
Control	0	47.23±1.27
Fluoxetine	10	10.01±0.74
CEE	20	23.12±2.70
CEE	40	19.52±1.58
CEE	80	12.79±0.46

**Values are recorded as means±SEM (n=5).
*Values are statistically significant ($p<0.05$) in relation to control. One-way ANOVA follow by Newman-Keuls Multiple Comparison tests

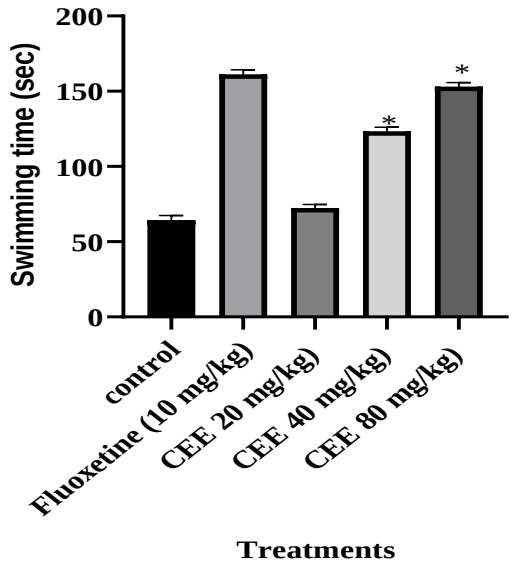

Figure 4: Effect crude ethanol extract on swimming times in mice.
Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

DISCUSSION

In the present study, the anxiolytic and antidepressant-like effects of the extract of Ficus platiphylla stem bark were studied in different experimental models. The Ficus platiphylla stem bark extract was first studied using the open field which gives a better indication of the animal's emotional state.

Animal found himself in a new environment such as open field become fearful as a result of over stimulation of sympathetic nervous system and consequently, there is increase in frequency of rearing, grooming and also, fecal production increases. These behavioral indices were ameliorated by anxiolytics and augmented by anxiogenic agents.[16,17] The number of squares crossed by diazepam and F. Platiphylla extract treated groups increased significantly when compared to control. The increased in Grooming Behavior due to fear in rodent when expose to a novel environment was reported to be taken as an index of behavioral adaptation to unpleasant condition.[18] In an open-field test anxiolytic agent decreases grooming as previously described[19] and in the present experiments, groups receiving F. platiphylla did reduce grooming. There was a significant reduction in the grooming time and an increase in the time spent by the mice at the center of the field in the extract and diazepam treated groups. Rearing and the number of line crossed was used in this study to evaluate the effect of F. platiphylla on spontaneous activities of mice. Increase in frequency of rearing and the number of line crossed was reported to be used as an index of the locomotor activity and indication of central nervous system stimulant properties respectively.[20] There was significant increase in rearing frequency and the number of squares crossing in ethanol extract treated groups.

These results taking together indicate that, in contrast to diazepam, the ethanol extract of Ficus platiphylla showed anxiolytic-like effects without affecting locomotor activity or without producing central nervous system depression. However, the animals treated with diazepam significantly decreased the number of line crossed by the animal suggesting a sedative effect at the dose used.

Elevated plus maze is one of the experimental model used to evaluate or screen anxiolytic agents, in which the animal try to avoid an open areas produced by the maze which may put them into aversive condition.[21,22] A significant increase in the duration and number of open arm entries display by the animal is an index of anxiolytic agents. There was a significant increase in the number of open arm entries and the duration in the present study, both the extract and the standard drug (diazepam). Flumazenil, a benzodiazepine receptor antagonist nullified significantly the anxiolytic effect induced by the F. platiphylla extract and the diazepam. This study therefore shows that the F. platiphylla stem bark may probably work via GABA-benzodiazepine pathway. Similarly, Pindolol also blocked the anxiolytic effect of the ethanol extract of F. platiphylla stem bark significantly. It was reported that Pindolol was reported to have blocked 5-HT1A autoreceptors as described previously according to.[23] Therefore, the anxiolytic-like effect of F. platiphylla may also be mediated by the 5-HT1A receptor.

The present study also characterized the effects of the ethanol extract of Ficus platiphylla stem bark, on mice performance in forced swimming test. Mice displayed inability to move when subjected to unpleasant situation such as forced swimming and is believe to reflect a state of despair, indicating depressive disorders in humans as previously described[12] and treatment with antidepressant drugs brought about a reduction in immobility time according to.[24]

It was reported that fluoxetine brought about a decrease in immobility characterized by an increase in swimming but did not have effect on duration of climbing.[25] It was

reported that fluoxetine, a selective serotonin reuptake inhibitor influence swimming in mice while tricyclic antidepressants affect the climbing behavior and drugs with selective effects on catecholamine transmission.[26,27]

Looking at it from the aforementioned report, the results obtained in this study strongly suggested the implication of the serotoninergic and catecholaminergic pathway in the antidepressant effect of F. platiphylla stem bark extract. In conclusion, our results revealed that F. platiphylla stem bark may possess some phytoconstituents with anxiolytic and antidepressant properties.

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