

## Original Article

# Anxiolytic and antidepressant effects of methanol stem bark extract of *Bombax buonopozense* P. Beauv. (Bombacaceae)

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### ABSTRACT

The aim of this study was to investigate the anxiolytic and antidepressant effect of stem bark of *Bombax buonopozense* P. Beauv. (*Bombacaceae*) in mice. Fresh dried stem bark of *B. buonopozense* was extracted with methanol by maceration which yielded 10.2% methanol extract. Anxiolytic and antidepressant effects of the methanol extract at 250, 500, and 1000 mg/kg were evaluated in mice using open-field test (OFT), hole-board test (HBT), forced swim test (FST), and tail suspension test (TST) models, respectively ( $n = 120$ ), were used in the four models, five groups were made each containing six mice, in each model. Group I received distilled water 10 ml/kg, Group II, III, and IV received 250, 500, and 1000 mg/kg of the *B. buonopozense* extract, respectively, while Group V received diazepam 0.5 mg/kg in OPT, HBT, and imipramine 10 mg/kg in FST, TST as positive controls in each of the models. *B. buonopozense* extract (1000 mg/kg) has significantly increased the number of square crossing in OFT compared with distilled water-treated group 10 ml/kg ( $P < 0.05$ ). However, *B. buonopozense* extract (500 mg/kg) shows a significant decrease in duration of immobility in FST compared to distilled water-treated group 10 ml/kg ( $P < 0.05$ ). This same extract did not show significant effect in HBT and TST models. Based on the results obtained in this research, *B. buonopozense* methanol extract can suppress symptoms of anxiety and depression in mice.

**Keywords:** Antidepressant, anxiolytic, *Bombax buonopozense*, extraction

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## INTRODUCTION

About 450 million people worldwide suffer from a mental or behavioral disorder (such as anxiety and depression), yet only a small number receive the most basic treatment.<sup>[1]</sup> This quantifies to 12.3% of the global burden of disease and may increase to 15% by 2020.<sup>[2]</sup> Researchers have now focused on search for novel therapeutic compounds for the treatment of neurological disorders. Plant materials are indispensable in this approach. Medicinal plant research is progressing globally, and this constantly demonstrates the pharmacological efficacy and safety of different plant species on various animal models.<sup>[3]</sup>

*Bombax buonopozense* is a large tropical tree that grows up to 40 m in height with large buttress roots that can spread up to 6 m. The individual leaflets have entire margin measuring from 8 to 23 cm in length by 3 to 7.5 cm in width. The undersides of the leaflets are either glabrous or puberulous and conical buds

which contain many seeds that are 5–6 mm in length, all of which have a cotton-like fiber covering.<sup>[4]</sup> The plant is widely distributed in African countries such as Nigeria, Ghana, Sierra Leone, and Uganda, and different parts are used for different purposes.<sup>[5]</sup> Some uses of *B. buonopozense* include antiulcer,<sup>[6]</sup> antimicrobial agent,<sup>[7]</sup> and antidiarrheal.<sup>[8]</sup>

## MATERIALS AND METHODS

### Plant Material and Preparation of Extract

Stem bark of *B. buonopozense* was collected on March 20, 2017, at Mashayar maiki village, Tureta local government area of Sokoto state, North western Nigeria. The plant was authenticated by Dr. Halilu Mshelia of the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher number PCG/UDUS/*Bombax*/0001 was assigned and kept at the Departmental Herbarium. The stem bark was

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shade dried at room temperature and size reduced to dry powder after which it was macerated with 70% methanol in water for 4 days with constant shaking. The resultant mixture was filtered using Whatman (No. 1) filter paper, and the filtrate dried using oven set at 39.9°C for 5 days.

### Drugs

Diazepam (Juhel Nigeria limited) and methanol (Sigma-Aldrich) extract of *B. buonopozense*, diazepam, imipramine (Dony Triumph and company, Nigeria Limited), and distilled water were used in this experiment.

### Animals and Treatments

All experiments were conducted in accordance with international standards of animal welfare recommended by the Society for Neuroscience (USA). The experimental protocol was approved by the Institutional Research Committee, Department of Pharmacology and Toxicology, UDUS. The minimum number of animals and duration of observation required to obtain consistent data were employed.

A total of 120 mice of both sexes weighing 17–23 g were used for these experiments, 30 for each model. The animals were maintained in a well-ventilated room with free access to food and water and divided into five groups ( $n = 6$ ). Group I, II, III, IV, and V received oral treatment of 10 ml/kg distilled water, 1000 mg, 500 mg/kg, 250 mg/kg *B. buonopozense* extract, and 0.5 mg/kg diazepam, respectively, for both open-field test (OFT) and hole-board test (HBT). The remaining 60 mice were separately used for forced swim test (FST) and tail suspension test (TST) (30 mice for each test). The animals were similarly divided as mentioned earlier ( $n = 6$ ). Similar dosing regimen was adopted as above, except that imipramine 10 mg/kg was used as the positive control in these models.

### OFT

The open-field area was made of acrylic transparent walls and black floor (30 cm × 30 cm × 15 cm) divided into nine squares of equal area. The open field was used to evaluate the exploratory activity of the animal.<sup>[9]</sup> The observed parameters were the number of squares crossed (with the four paws) and number of rearing.

### HBT

According to this model, the exploratory behavior of the mice is measured by determining the number of head dipping inside the holes which reflect no anxious behavior and less ability to acclimatize the arena, and decreased number of head dipping inside the hole reflects anxious behavior. Hole board apparatus consisted of an enclosed 50 cm × 50 cm arena made of white opaque Plexiglas with a raised floor (5 cm above a white opaque Plexiglas subfloor) containing four equidistant holes (4 cm in diameter). Each hole center was 10 cm from the two nearest walls so that holes were equidistant from adjacent corners.<sup>[10]</sup>

### FST

According to this model, if animal is exposed to chronic stress and fails to escape, it gradually loses its hope to escape, stops its activity and mobility, and becomes frustrated and immobile. To measure the immobility time, all time intervals when mouse is immobile are recorded within a specified duration. Longer duration of immobility mimicked depression and shortening duration of immobility indicates efficacy of antidepressant employed. In this test, a glass container that has a length of 25 cm, a width of 12 cm, and a height of 15 cm is filled with 25°C water and the mouse from a 20-cm distance to the water surface is slowly placed in the water. Conventionally, lack of paddling is considered immobility. All chronological measurements were conducted by one person. Total FST lasts for 7 min and the first 3 min is specified for adaptation to test condition, and therefore, immobility time during this period is not recorded. All tests of variables were recorded by one person.<sup>[11]</sup>

### TST

In the TST used to assess the extent of depression, metal bars of 70 cm high are used, a 50 cm rope is longitudinally stretched between them, and mouse tail is closed by the rope and the mouse is hanged up from its tail. When mouse becomes completely inactive and exhibits no reaction, it is considered immobile. The total duration of TST, as with FST, is 6 min and the first 2 min is specified for animal's adaptation to the apparatus. The immobility time (s) in the following 4 min was recorded by chronometer. All tests of variables were recorded by one person.<sup>[11]</sup>

### Data Analysis

Statistical analysis of the results was carried out using one-way analysis of variance followed by Dunnett's *post hoc*. Data were expressed as mean ± standard error of mean and  $P < 0.05$  was considered statistically significant.

## RESULTS

### OFT

The effect of oral administration of diazepam 0.5 mg/kg and three different doses of *B. buonopozense* 250, 500, and 1000 mg/kg on central and peripheral square crossing with four paws is illustrated in Figures 1 and 2, respectively. As illustrated, there was significant ( $P < 0.05$ ) increase in central square crossing on administration of 1000 mg/kg *B. buonopozense* extract compared to group receiving 10 ml/kg distilled water. However, there is no significant increase in central square crossing with groups treated with 250 and 500 mg/kg of *B. buonopozense* extract when compared with the control group.

### HBT

Figure 3 illustrates the effect of oral administration of diazepam 0.5 mg/kg and *B. buonopozense* methanol extracts (250, 500,

and 1000 mg/kg) on head dipping of the mice. As illustrated, there is no significant increase in number of head dipping on administration of *B. buonopozense* methanol extract (250, 500, and 1000 mg/kg) as compared with the groups treated with distilled water 10 m/kg ( $P < 0.05$ ). The positive control group receiving 0.5 mg/kg diazepam shows a significant increase in number of head dipping when compared with negative control group receiving distilled water 10 ml/kg ( $P < 0.05$ ).

### FST

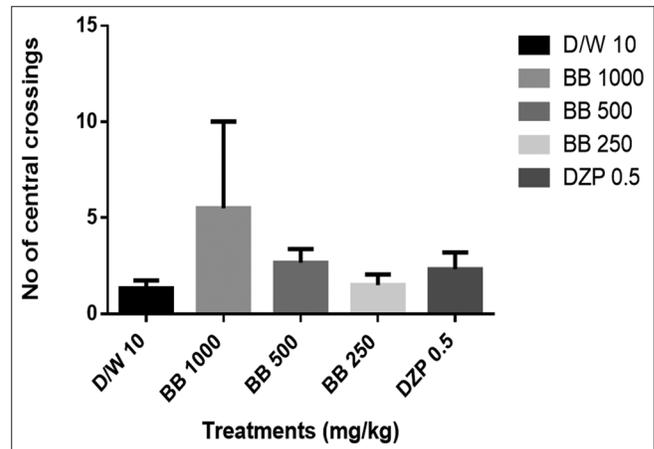
Figure 4 illustrates the effect of oral administration of imipramine 10 mg/kg and *B. buonopozense* methanol extracts (250, 500, and 1000 mg/kg) on the duration of immobility in the FST in mice. As illustrated, immobility time was increased in distilled water-treated group when compared with the test groups and positive control group. There was a significant ( $P < 0.05$ ) decrease in duration of immobility at 1000 mg/kg dose of *B. buonopozense* compared to group treated with distilled water 10 ml/kg. Methanol extract of *B. buonopozense* at doses of 500 and 250 mg/kg shows no significant decrease in the duration of immobility compared to distilled water-treated group.

### TST

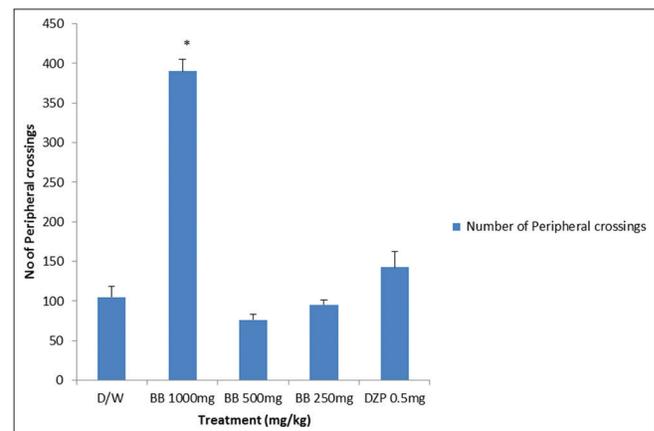
Figure 5 illustrates the effect of imipramine and different doses of *B. buonopozense* (250, 500, and 1000 mg/kg). All the doses of *B. buonopozense* produced no significant decrease in duration of immobility when compared with the negative control group receiving distilled water 10 ml/kg. *B. buonopozense* dose of 1000 mg/kg has produced a decrease in duration of immobility, but not statistically significant decrease when compared with distilled water group 10 ml/kg ( $P < 0.05$ ).

## DISCUSSION

This research investigated the effect of *B. buonopozense* stem bark methanol extract (250, 500, and 1000 mg/kg) on symptoms of depression and anxiety induced by unpredictable stress and exploratory behavior in novel arena, respectively. Oral administration of *B. buonopozense* (1000 mg/kg) to mice in new arena of open field shows significant increase in number of central and peripheral square crossing when compared with distilled water-treated group, this intense expression of exploratory behavior indicates anxiolytic activity.<sup>[12]</sup> This finding was consistent with other author's findings that revealed that plant extract containing flavonoid, specifically apogenin which was found binding the central benzodiazepine receptor possessed anxiolytic activity.<sup>[13,14]</sup> Diazepam was also observed to increase the number of central and peripheral square crossing of mice in the OFT, similar finding with diazepam in OFT was reported by Sousa *et al.*<sup>[15]</sup> and Kłodzińska *et al.*<sup>[16]</sup> Diazepam potentiates GABA-mediated inhibition through increase in the affinity of this inhibitory neurotransmitter to its recognition sites within the GABA<sub>A</sub> receptor complex, by increasing the opening frequency of the chloride ion channel which leads to



**Figure 1:** Effect of diazepam and different doses of methanol extract of *Bombax buonopozense* stem bark on central square crossing in open-field test, differences were considered statistically significant at  $P < 0.05$ . D/W - distilled water, DZP - diazepam

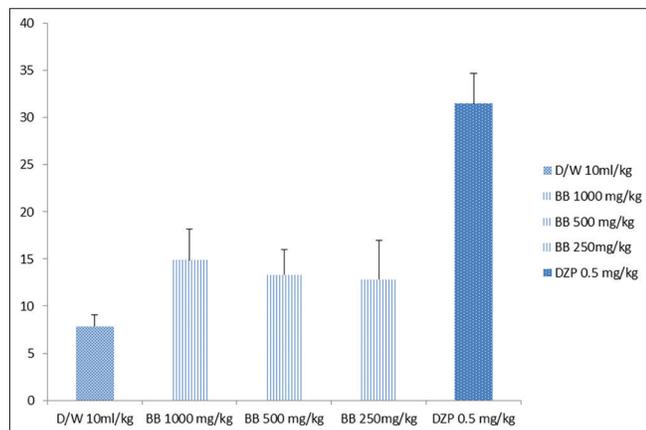


**Figure 2:** Effect of diazepam and methanol extract of *Bombax buonopozense* stem bark on peripheral square crossing in open-field test, differences were considered statistically significant at  $P < 0.05$ . BB - *Bombax buonopozense*, D/W - Distilled water, and DZP - Diazepam

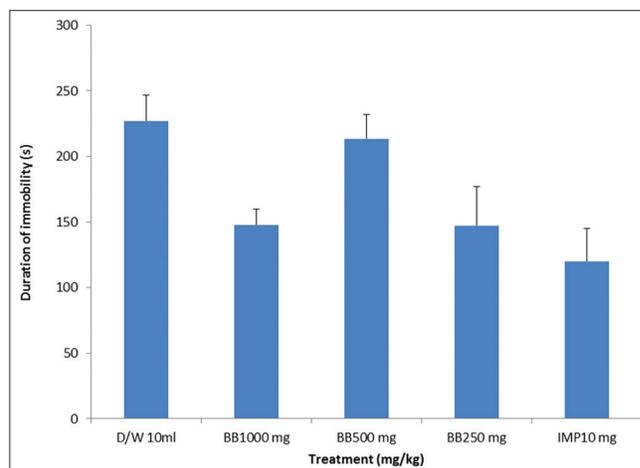
the enhancement of influx of chloride anions into the neuron and subsequent hyperpolarization.<sup>[17]</sup>

The extract produces an increase in exploratory behavior by increasing the number of head dipping inside the hole. The hole-board experiment is a measure of exploratory behavior in animals.<sup>[18]</sup> An increase in exploratory activity in mice as demonstrated by increase in head dip is a measure of anxiolysis. This was in agreement with the findings of Onasanwo *et al.*<sup>[19]</sup>

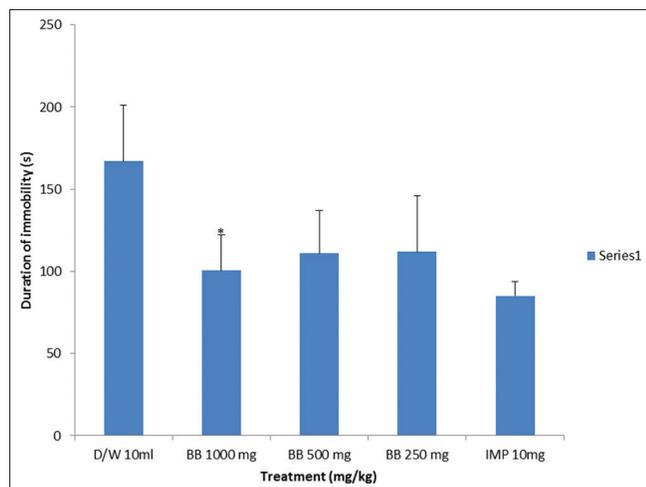
From the result obtained in the FST, the effect of imipramine (10 mg/kg) and *B. buonopozense* (250, 500, and 1000 mg/kg) on chronic unpredictable stress-induced immobility was determined. Mice treated with *B. buonopozense* (1000 mg/kg) showed significant decrease in duration of immobility in forced swim



**Figure 3:** Effect of diazepam and different doses of methanol extract of *Bombax buonopozense* stem bark on number of head dipping in hole-board test, difference is considered statistically significant at  $P<0.05$ . BB - *Bombax buonopozense*, DZP - Diazepam



**Figure 5:** Effect of imipramine and different doses of *Bombax buonopozense* on the duration of immobility in tail suspension test in mice, differences were considered statistically significant at  $P<0.05$ . Imip - Imipramine



**Figure 4:** Effect of imipramine and different doses of *Bombax buonopozense* on the duration of immobility in forced swim test, differences were considered statistically significant at  $P<0.05$  BB - *Bombax buonopozense* IMIP - Imipramine

mice compared to distilled water-treated group. This significant decrease in immobility duration in forced swim mice indicates antidepressant activity.<sup>[20]</sup> The decrease duration of immobility exhibited by *B. buonopozense* (1000 mg/kg) is comparable to imipramine, a standard antidepressant drug.<sup>[21]</sup> This corroborates the finding of Porsolt *et al.*<sup>[20]</sup> and Cryan *et al.*<sup>[21]</sup>

The effect of *B. buonopozense* extract on the duration of immobility in the TST further buttresses the antidepressant activity of the plant. The characteristic behavior assessed in this test, termed immobility, has been considered to reflect behavioral despair similar to that seen in human depression, and it is well known that antidepressant drugs are able to reduce the immobility time in rodents.<sup>[22]</sup>

The combined effects observed in both models further provide scientific basis for the traditional application of *B. buonopozense* in the management of anxiety/depressive disorders. However, further studies are needed to confirm our findings and establish its exact mechanism of antidepressant and anxiolytic activity.

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