

## Anxiolytic-like Effect of Crude Methanolic Extract of *Feronia limonia* and Rose Petals

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**ABSTRACT:** *Natural lead compounds are one of the prime sources of novel drug discoveries. The aim of the study was to investigate the Anxiolytic-like action of Feronia limonia (L.) and Rose petals in order to understand the scientific basis for their usage. Long Evans Rat was used for the study. Crude methanolic extracts of Feronia limonia and Rose Petals were used along with standard diazepam 1mg/kg. In the elevated plus maze test (EPM) Feronia limonia and Rose Petal extract showed POEA (percentage of entries into open arms) 54% and 47% respectively. In terms of PTOA (percentage of time in the open arms) Feronia limonia was 45% and Rose Petal was 49%. In the open field test, both the extract showed almost similar results in terms of the number of squares crossed, grooming, and rearing. Exploratory behaviour (hole-board test) was measured by the number of head dips which was 54 for Feronia limonia, 51 for Rose Petal, and 67 for diazepam. Tail suspension test showed an almost similar effect compared to diazepam. Overall crude methanolic extract of Feronia limonia (L.) and Rose petals showed comparable anxiolytic-like effects.*

**Keywords:** *Anxiolytic, diazepam, extract.*

### 1. INTRODUCTION

Limonia [*Feronia Limonia* (L.) Swingle] is a deciduous, slow-growing, erect tree up to 12 meters tall that belongs to the Rutaceae family. It is a fruit-bearing tree native to India, found in arid warm parts of Burma and Sri Lanka, and widely planted across Southeast Asia and Northern Malaya [1]. In comparison to many other fruits, the wood apple is a nutrient-dense fruit [2]. Limonia fruit is high in carbs, fibers, protein, vitamins, and minerals, according to physicochemical analysis [3, 4, 5, 6, 7, 8, 9, 10]. According to Kamalakkannan and Prince [11], the aqueous extract of the

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Limonia fruit pulp has antioxidant properties, and daily oral administration of the aqueous extract of Limonia fruit (125 and 250 mg/kg) twice a day for 30 days to diabetic rats reduced fasting blood glucose and glycosylated hemoglobin levels. Limonia fruit pulp is rich in carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids, terpenoids, coumarins like aegeline, aegelenine, marmelin, o-mthethyl halfordinol, alloimperatorin, furocoumarins, psoralen, oisopentenyl halfordinol, marmelosin, linoleic acid, tannins, phlobatannins, flavon-3-ols, leucoanthocyanins, anthocyanins, and flavonoid glycosides [7, 8, 12, 13, 14].

Rosa (R.) species have been used as herbal medicine for dysmenorrhea, diarrhea, and nephritis, as well as for enhancing blood circulation, pain management, hemostasis, skin anti-inflammatory, and hemorrhoids [15, 16, 17, 18]. Roses have been postulated to have distinct biological activities due to the presence of numerous terpenes (glycosides), flavonoids, and anthocyanins [19, 20, 21, 22]. Indeed, Kumar [23] found that methanolic extracts of *R. brunonii*, *R. bourboniana*, and *R. damascena* have high antioxidant activity. Anxiolytics are currently among the most frequently prescribed drugs worldwide, particularly in the Western world, and specifically the most prescribed psychoactive drugs [24]. Because there is no experimental evidence on the suspected anxiolytic action of Limonia and Rose Petal, it seemed prudent to investigate their effects on anxiogenic situations in order to understand the scientific basis for their use.

## **2. METHODS AND MATERIALS**

### ***A. Animals***

Long Evans Rat (58-64g) of either sex was used for the study. The animals were housed in groups of six in polypropylene cages, under standard laboratory conditions of temperature ( $25 \pm 2^\circ\text{C}$ ), lighting (800–2000 h), and relative humidity ( $55 \pm 5\%$ ). The animals were fed standard food and water. The animals were maintained for a minimum of seven days to acclimate to a new environment or circumstance. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) Department of Pharmacy, UITS.

### ***B. Plant material and preparation of the extract***

Fresh, juicy Limonia fruits and rose petals were purchased at a local market in Rajshahi. The pulp and seeds were manually removed, and the rose petals were separated from the flower before being oven-dried overnight at  $45^\circ\text{C}$ . The dried materials were powdered using a grinder. For

the extraction, 50gm of the sample was used at the desired concentration of methanol, temperature, and solvent-to-liquid ratio as per the experimental plan. The extraction was carried out in a refrigerator circulatory bath for 3h at 100 rpm and 45<sup>0</sup> C. At room temperature, the extracts were dried. Prior to the investigation, both the dried extract and the filtrates were stored in the refrigerator.

### ***C. Drugs and Doses***

Crude methanolic extract of Limonia and rose petal were emulsified with 0.25% Tween 80(Sigma-USA) and dissolved in distilled water. Animals were treated with the substance at doses of 400 mg/kg, intraperitoneally (IP), 60 mins before the experiments. Controls received vehicle (Water and 0.25%Tween 80) at the same volume (10 ml/kg) administered by the same route as the treated groups. Diazepam (DZP) 1 mg/kg was used as standard and intraperitoneally injected after dissolved in distilled water. It is well known that benzodiazepines act as anxiolytics (at low doses) and also produce sedation and myorelaxant effect at higher doses [25]. So, we used diazepam at 1 mg/kg in the Elevated plus maze test (EPM) and hole-board tests as standard drug for anxiolytic effect.

### ***D. Experimental protocol***

The animals were tested and examined in a closed room at a constant temperature (25±2 °C) during light time. All experiments were carried out on various days and with distinct groups of animals [26].

### ***E. Elevated plus maze test (EPM)***

The elevated plus maze test for rats [27] consisted of two perpendicular open arms (30×5 cm) and two closed arms (30×5×25 cm) also in a perpendicular position. The open and closed arms were connected by a central platform (5×5 cm). The platform and lateral walls of the closed arms were constructed of clear acrylic, while the floor was composed of black acrylic. The labyrinth was 45 centimetres above the ground. Following treatment, the animal was put in the centre of the plus maze, nose to one of the closed arms, and watched for 5 minutes, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The time of permanence is the amount of time the animal spends in open and closed arms. Anxiolytic compounds reduce the animal's aversion to open arms and promote the exploration thereof. The parameters observed were: percentages of entries into open arms (PEOA), number of entries in the open arms (NEOA), time

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of permanence in open arms (TPOA), and percentage of time of permanence in the open arms (PTOA). To this test, the animals were divided into four groups of 5 animals each. The different groups were treated with: Water (control), *Feronia limonia* 400 mg/kg and rose petal 400 mg/kg, and Diazepam 1 mg/kg.

### **F. The hole-board test**

The hole-board test for exploratory behavior in rats was used as described previously by Clark [28]. The apparatus used was a wooden board of 60×30 cm with 16 evenly spaced holes. In brief, adult male rats were randomly divided into five groups with 4 rats per group. Three groups received 400 mg/kg, IP of *Feronia limonia* and Rose petal crude extract respectively. One group received DZP (1 mg/kg, IP) as standard and the remaining group (control) received Water.

### **G. Tail suspension test**

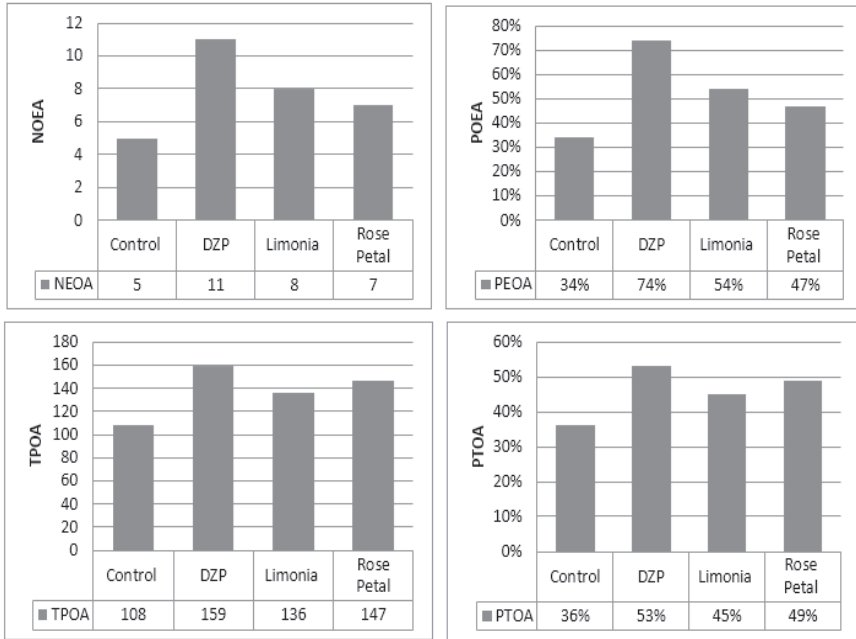
The tail suspension test has been described by Steru [29]. Long Evans Rats were housed in plastic cages with a 12 h light cycle with food and water freely available. For the test, the animals were divided into four groups with five animals per group. The different groups were treated with: a line (control), *Feronia limonia* 400 mg/kg and Rose petal 400 mg/kg, diazepam 1 mg/kg, in accordance with the described method [29]. They were suspended by adhesive tape 1 cm from the tip of the tail on the edge of a shelf 58 cm above a table top. The length of immobility is measured for 5 minutes.

## **3. RESULT & DISCUSSION**

### **A. Elevated plus maze test (EPM)**

The peritoneal administration of the methanolic extract of *Limonia* and *Rose petal* (400 mg/kg) produced a significant increase in the time spent in the open arms as well as the number of entries in the open arm of the elevated plus maze indicating the anxiolytic activity of the drugs. Vehicle treated rat spent 108 s in the open arm. Rat treated with methanolic extract of *Limonia* and *Rose petal* spent 136 s and 147 s in the open arm respectively. The number of open arm entries also increased to 7 and 8 for *Rose petal* and *Limonia*, respectively, as compared to the vehicle (5). Vehicle treated rat spent 36% in the open arm. Rat treated with methanolic extract of *Limonia* and *Rose petal* spent 45% and 49% s in the open arm respectively. The number of open arm entries also increased to 47% and 54% for *Rose petal* and *Limonia*, respectively, as compared to the vehicle (34%). The administration of diazepam (1.0 mg/kg i.p.)

significantly increased the number of entries, percentage of entries in the open arms, time permanence in the open arms as well as the duration of stay in the open arms, as compared to the extract treated groups. The results are given in Figure 1.

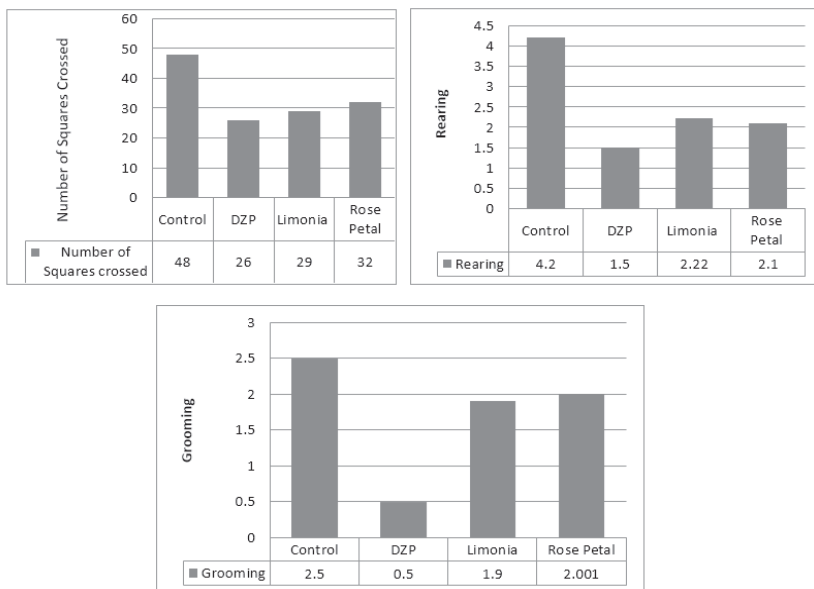


**Figure 1:** Elevated plus maze test of groups of rats which received vehicle, Limonia, Rose Petal (400mg/kg), diazepam (DZP 1 mg/kg). The parameters analysed were: NEOA: number of entries into open arms; PEOA: percentage of entries into open arms; TPOA: time of permanence in the open arms; and PTOA: percentage of time in the open arms.

**B. Open-field test**

The oral administration of methanolic fractions significantly increased the rearings (2.1 for Rose petal and 2.22 for Limonia), self-groomings (2.001 for Rose petal and 1.9 for Limonia) and number of squares crossed (32times for Rose petal and 29 times for Limonia). Diazepam also induced significant anxiolytic activity and the effects were found to be highly significant and more than that of the extracts. The aqueous fractions did not produce significant changes, indicating a lack of anxiolytic activity. The results are shown in Figure 2.

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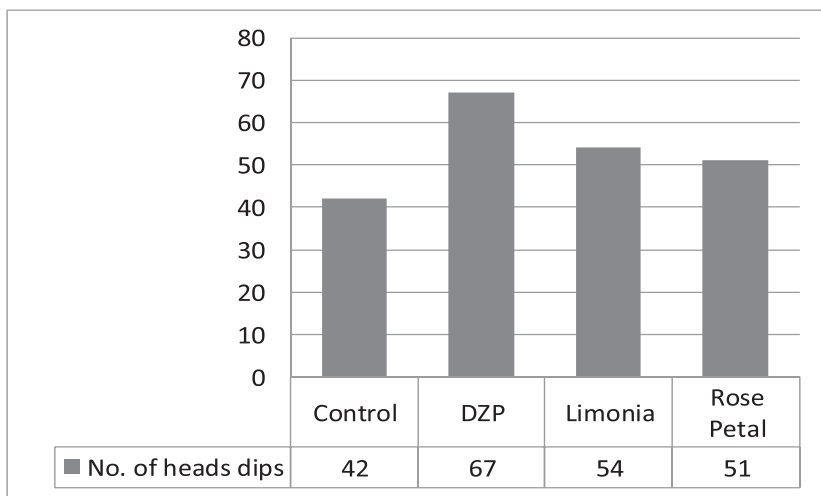


**Figure 2:** Rats were divided into groups and tested in the open field with the vehicle, Limonia and Rose Petal (400 mg/kg), and diazepam (DZP 1 mg/kg). The number of squares crossed, grooming, and rearing were the factors analyzed.

**C. Hole-board test**

Rats were tested using the hole-board method following Clark’s procedure (28). The tool utilized was a Wood and Cartoon measuring 60 x 30 cm with 16 uniformly spaced holes. In a nutshell, adult male rats were split into five groups randomly. Limonia and Rose Petal were administered in three groups at varying levels (400 mg/kg IP). DZP (1 mg/kg, IP) was administered to one group as a standard, while the prepared solution was administered to the other group (the control). The number of head dips into the holes for each animal was counted for five minutes after the administration of DZP, and thirty minutes and sixty minutes following the administration of Limonia and Rose Petal.

In the hole-board test, DZP (67) had the most rats dip their heads compared to the control (42), Limonia (54), and Rose petal (51). When compared to the standard and the control, the performance of the plant extract is noteworthy.



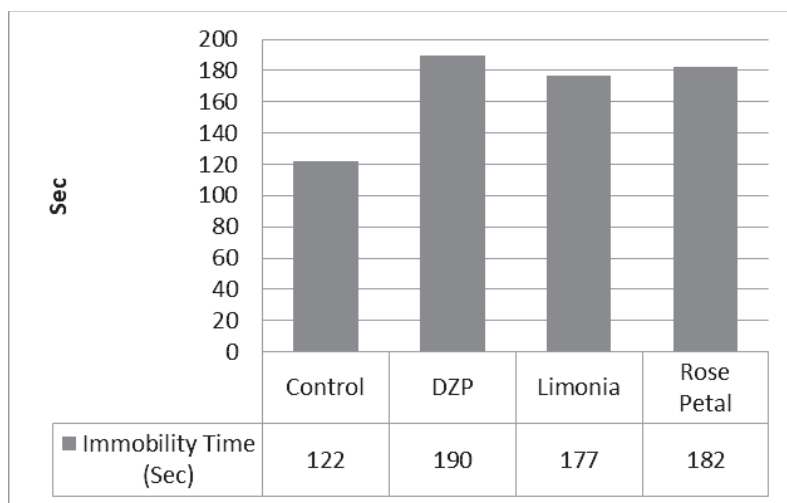
**Figure 3:** Hole-board test of groups of rats which received vehicle, Limonia, Rose Petal (400 mg/kg), and diazepam (DZP 1 mg/kg). The parameters analysed were: Number of head dips.

**D. Tail suspension test**

Steru has provided a description of the tail suspension test [29]. Long Evans Rats were kept in 12-hour light cycles in plastic cages with free access to food and water. The animals were split into four groups for the test, each with five animals. A line (control), *Feronia limonia* 400 mg/kg and Rose petal 400 mg/kg, as well as diazepam 1 mg/kg, was administered to the various groups. They were placed on the edge of a shelf 58 cm above a table-top, suspended by adhesive tape 1 cm from the tip of the tail. Five minutes are used to quantify the duration of immobility.

Two groups received Limonia and Rose Petal (400 mg/kg IP) in this test (Fig. 4). Rats in the control group spent less immobilizing than those in the other groups, which received a prepared solution instead of the normal dose of DZP (1 mg/kg, IP) in the first group. DZP (190) has the longest immobility time on the graph, whereas Control has the shortest immobility time (122). Rose petals (182) and Limonia (177) exhibit a modest variation in their activity.

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**Figure 4:** Tail suspension test of groups of rats which received vehicle, Limonia, Rose Petal (400 mg/kg), and diazepam (DZP 1 mg/kg). The parameters analysed were the immobility time.

#### 4. CONCLUSIONS

In many behavioral animal models, including the open-field, elevated plus-maze, hole-board, and tail suspension test, the effects of methanolic extract of Limonia and Rose petals were investigated. These are animal models used in preclinical pharmacological testing of actions on the central nervous system that shed new light on how drugs affect anxiolysis, motor function, and psychomotor performance. Based on our research, mangiferin can be considered a potential biomarker. As it has been previously documented for its antioxidant properties, Limonia and Rose petals were given at a dose of 400 mg/kg I.P. [Kamalakkannan and Prince [11]. In the current trials, Limonia and Rose petals showed anxiolytic efficacy similar to that of the GABA agonist diazepam. Involvement of a similar mechanism i.e. GABAergic activity of the extracts and the isolate cannot be ruled out. Investigating GABA antagonists' ability to inhibit the observed anxiolytic action will be beneficial in the hope that they will support the proposed mechanism.

#### 5. RECOMENDATIONS

Further study can be done with specific lead compound of the crude extracts.



## 6. ACKNOWLEDGEMENT

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