EVALUATION OF ANXIOLYTIC ACTIVITY OF Ficus pumila L. LEAF EXTRACT IN EXPERIMENTAL ANIMALS

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ABSTRACT
The objective of this study was to investigate the anxiolytic activity of ethanolic extract of leaves of Ficus pumila L. (Moraceae) in experimental animals. Two doses (200 mg/kg and 400 mg/kg) of the extracts were used for the study. The experiments was performed by using three models such as elevated plus maze (EPM), open field test and Y maze model. Diazepam 2mg was used as the standard drug. All drugs were administered by the oral route. The result showed that administration of the extract of Ficus pumila L. to the animals significantly increases the both number and time spended in open arms in the elveted plus maze test. In open field the animals showed an increase in the ambuluation, total locomotion, central locomotion and decrease in latency, rearing and time taken to enter central compartment. The results obtained in the Y-maze model showed that the numbers of visits of mice in the three arms were found to be decreased significantly in a dose dependent manner for all extract treated groups when compared to the control animals. Phyto-chemical analysis revealed the presence of carbohydrate, glycosides, sterols, flavonoids and triterpenes. From the results obtained it could be concluded that the ethanolic extract of leaves of Ficus pumila L. possess significant, dose dependent anxiolytic activity.

KEYWORDS
Anxiolytic activity, Ficus pumila L, Elevated plus maze, Open field and Y-maze

INTRODUCTION
Anxiety is an emotional state, unpleasant in nature, associated with uneasiness, discomfort and concern or fear about some defined or undefined future threat. Some degree of anxiety is a part of normal life. Treatment is needed when it is disproportionate to the situation and excessive. Some psychotic and depressed patients also exhibit pathological anxiety1. Anxiety can affect anyone, whatever their age, gender etc. It affects our thoughts, physical...
reactions, moods and behaviours. Anxiety can also cause us to feel panicky and frightened and prevent us from doing things. Too much stress in our lives can result in higher levels of anxiety.

New epidemiological evidence suggests that they are much more prevalent than traditionally thought, affecting up to half of the general population\(^2\). Anxiety affects one of eight of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade\(^3\). So development of new anxiolytics has been an area of interest. Many secondary plant metabolites has been reported in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system such as noradrenalin, serotonin, gamma-amino butyric acid (GABA), benzodiazepine (BDZ) neurotransmitters activities which is believed to be involved in the development of anxiety. Major drug classes for the treatment of anxiety disorders are Benzodiazepines (BZDs), Selective serotonin-reuptake inhibitors (SSRIs), Tricyclic antidepressant, \(\beta\)-blockers and Azapirones\(^4\). All this drug classes currently used are associated with side effects that very occurrence and severity. Like They may produce undesirable effects such as drowsiness, ataxia, and sedation, muscle relaxation, insomnia, hepatotoxicity and in addition they adversely interact with other CNS depressants, particularly alcohol\(^5,6\). For this reason; many researchers have been evaluating new compounds from herbs with fewer undesirable effects. It has been suggested that various traditional herbal medicines also possess anxiolytic activity such as *Bacopa monnieri* Linn, Ginseng, *Ginkgo biloba* Linn, *Piper methysticum* forst and *Salvia officinalis* Linn. Research has also focused on the development of drugs with fewer side effects, such as sedation, muscle relaxation, and drug dependence. Herbal medicine is fast emerging as an alternative treatment to synthetic drugs for treatment of most diseases possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness and plants are more potent healers because they promote the repair mechanisms in the natural way\(^7\). In the present study a plant from the Genus *Ficus*, named *Ficus pumila* L. of the Moraceae family was selected, which is a scandent shrub with evergreen coriaceous leaves that is normally grown between the trees as well as on fragmented surface. The leaves of the plant has been traditionally consumed by some Okinawan elders either as a beverage or used as an invaluable medicinal herb by the folks to treat diabetes, dizziness, high blood pressure, and neuralgia\(^8,9\). Several studies have been performed on the composition of *Ficus pumila* L. and phytochemical analysis was performed and confirmed the presence of carbohydrate, glycosides, sterols, flavonoids and triterpenes. The important constituents isolated in the previous study were apigenin, luteolin, rutin, genistein, hesperidin, astragalin, isoquercitrin, and chrysin\(^10\). Although many other species of this genus such as *Ficus hispida* Linn\(^11\), *Ficus exasperata* Vahl\(^12\), *Ficus religiosa*\(^13\) has been reported for anxiolytic activity. However, the anxiolytic activity of *Ficus pumila* L. had never been investigated, thus the present study was initiated to evaluate the anxiolytic activity of ethanolic extract of leaves of *Ficus pumila* L. in experimental animals.

**MATERIAL AND METHODS**

**Collection of Plant material and preparation of extract**

The leaves of *Ficus pumila* L. were collected from the campus of Nandha college institution- Erode (Tamilnadu). The plant was identified and authenticated by Botanical Survey of India, Tamilnadu Agricultural University Campus (TNAU), Coimbatore. The voucher specimen (BSI/SRC/5/23/2012-13/Tech-448) has been deposited in the herbarium of TNAU for future reference. The leaves were shade dried, powdered and were extracted using 70% ethanol as the solvent in a soxhlet apparatus until complete extraction. Solvent evaporation under reduced pressure was carried out to get semisolid extract which was used for the studies.
Experimental animal
The study was conducted on Wistar Albino rats of 150 - 200 g and Swiss Albino mice of 15 – 20 g maintained under standard conditions (room temperature 24\(^0\)C- 27\(^0\)C and humidity 60 – 65 %). The food in the form of dry pellets (M/s Hindustan Lever Foods, Bangalore) and water were available ad libitum. Rats and mice of either sex were selected and grouped in to four having 6 animals each. All the experimental procedures and protocols used in this study were reviewed by the InstitutionalAnimal Ethics committee (688/2/C-CPCSEA) of NCP and were in accordance with the guidelines of the IAEC. Approval was obtained from IAEC, NCP (Proposal No- NCP / IAEC / No: 9/2012-13).

The animals were divided into four groups, each containing six each. Group I was served as solvent control and received 0.5% CMC (1 ml/kg). Group II treated as positive control was received diazepam (2 mg/kg). Group III and IV were received ethanolic extract of *Ficus pumila* L. 200mg/kg and 400 mg/kg respectively. All the treatments were administered orally 60 min prior to start the experiment.

Phytochemical screening
The freshly prepared crude ethanolic extract of *Ficus pumila* L. was qualitatively tested for the presence of major phytochemical constituents according to standard methods\textsuperscript{14}.

ANXIOLYTIC ACTIVITY STUDIES

Elevated plus maze test\textsuperscript{15}
Adult wistar albino rats weighing 150 to 200g were used for the study. They were housed in clean, clear, polypropylene cages in groups of four and maintained at 24.0±2\(^0\)C with 12 hrs light and dark cycle and had free access to food and water ad libitum. Animals were kept in experimental lab for seven days prior to experiment to adapt the new laboratory conditions. Each rat was used only once.

Drugs and dosage
The test drug ethanolic extracts of *Ficus pumila* L., standard anxiolytic drug Diazepam were suspended in 0.5% carboxy methyl cellulose (CMC) was administered orally. Each drug solution was prepared freshly just prior to the administration. Drugs, dosage and number of animals used per treatment are shown in following table. Drugs/vehicle was administered 60 minutes prior to the experiment through oral routs. The animals are divided into four groups with six animals in each group (Table No.1).

The wooden maze consisted of two open arms (length 50 cm X breadth 10 cm) and two closed arms of the same size (height 40 cm). The arms of the same type were opposite to each other, with a central square of 10 cm. The maze was elevated to a height of 50 cm above the floor. Rats were individually placed on the centre the maze facing on open arm and observe the following for next 5 mints.

- Number of entries to the open arm
- Number of entries to the closed arm
- Time spend in open arm
- Time spend in closed arm

Open field Model\textsuperscript{16, 17}
The open field apparatus was made up of plywood consists of 56 x 56 (l x b) cm. The entire apparatus was painted black and 6 mm thick white lines divided the floor in to 16 square of identical dimension. Open field was lightening by 40 W bulb focusing on to the field from the height of about 100 cm. Swiss albino mice are used for this experiment. The animals were divided in to four groups of six in each group. The first group animals were treated with 0.5% CMC, the second group was treated with diazepam (2mg/kg) whereas 200mg/kg, and 400mg/kg of the test extract have been given to the third and fourth group of animals respectively. All drugs were administered by oral route. One hour after the drug treatment, each animal were placed at one corner of the apparatus and the following behavioural aspects were noted in the next 5 min.

**Latency**
- Time taken by animal to leave square in which it was placed
- Time taken to enter central compartment.
- Total locomotion and central locomotion.
- Ambulation: Number of square passed by animal
- Rearing: Number of times animal stood on its hind legs.

Y maze model\textsuperscript{18}
Y-maze is made of black painted wood or grey plastic. Each arm is 40 cm long, 13 cm high 3 cm
white at the bottom, 10 cm wide at the top and converges at an equal angle. Mice were treated with the extract of *Ficus pumila* L. (200 and 400 mg/kg p.o.) and vehicle for 7 days once daily p.o. and the last dose was given on the 7th day, 60 min before starting the experiment. The standard drug Diazepam was given at a dose of 2 mg/kg p.o., 60 min before starting the experiment. For a period of 5 min, the total numbers of visits to different arm were measured. After each trial, the Y-maze apparatus was wiped clean with ethanol (10%) solution.

**RESULTS**

**Phytochemical analysis**
The phytochemical analysis of the ethanolic extract of *F. pumila* L. revealed the presence of carbohydrate, glycosides, sterols, flavonoids and triterpenes.

**Elevated Plus Maze**
The results shows that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spend in the closed arms were decreased significantly in the extract treated groups. The magnitude of the anxiolytic effects of 200 mg/kg and 400 mg/kg of ethanolic extract of *Ficus pumila* L. was comparable to that of diazepam 2mg/kg p.o (Figure No.1 and 2). The results are shown in the Table No.2.

**Open Field Test (OFT)**
In the open field test (OFT), the animals treated with ethanolic extract of *Ficus pumila* L. was shows a significant increases in total locomotion, central locomotion and ambulation. At the same time the treated animals shows a significant decrease in latency, rearing and time taken to enter the central compartment. This observation of the animals in the open field indicates that the ethanolic extract of *Ficus pumila* L. have significant anxiolytic properties. The magnitude of the anxiolytic effects of 200 mg/kg and 400 mg/kg of alcoholic extract of *Ficus pumila* L. was comparable to that of diazepam 2 mg/kg p.o (Figure No.3 and 4). The results are given in the Table No.3.

**Y- maze test**
A significant decrease in the number of visits in the three arms of the Y-maze was observed in the Diazepam treated animals as compared to the control animals. Both the doses of ethanolic extract of *Ficus pumila* L. (200mg/kg and 400mg/kg) showed a significant decrease in the number of visits in the three arms of the Y-maze which was comparable with the standard Diazepam (2mg/kg) (Figure No.5). The results are given in the Table No.4.

**DISCUSSION**
Numerous animal models have been developed to study anxiolytic activity in order to prove the effectiveness of novel drugs, study their mechanisms of action, or evaluate the pathophysiological phenomena involved in anxiety. The Elevated plus Maze (EPM) is the first choice model for the evaluation of anxiolytic drugs and has been used for both rats and mice. The evaluation of the anxiolytic activity of *Ficus pumila* L. was performed with the elevated plus-maze (EPM), open field and Y-maze models. The prime measures in the EPM are the proportion of entries into the open arms and of the time spent on the open arms. An anxiolytic effect is suggested when the drug enhances open arms entries without altering the total number of arm entries. In the present study the rats were treated with extracts showed a significant increase of both entries and time spend in the open arms of the maze, similar to the effects observed after the reference anxiolytic drug diazepam. These results could indicate an anxiolytic-like activity to the ethanolic extract of leaves of *Ficus pumila* L.

The behaviour of experimental animals in the open field is used as a measure of exploration, anxiety, and locomotor behaviour. Animals when removed from their acclimatized cage and placed in a new environment express anxiety and fear, by showing changes in all or some parameters, such as decreases in ambulation and exploration, and immobilization or freezing due to increased autonomic activity. These paradigms are attenuated by standard anxiolytics, and potentiated by anxiogenic agents. In the present study it was noted that the animals...
treated with extracts shows an increase in ambulation, total locomotion, central locomotion and decrease in latency, rearing and time taken to enter central compartment. From this we can understand that the ethanolic extract of *Ficus pumila* L. have significant anxiolytic activity.

The results obtained in the Y-maze model showed that the numbers of visits of mice in the three arms were found to be decreased significantly in a dose dependent manner for all extract treated groups when compared to the control animals, which supports the anxiolytic activity of *Ficus pumila* L (Figure No.6).

**Table No.1: Drugs and dosage**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups(n=6)</th>
<th>Treatment</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.5% CMC(control)</td>
<td>1.0 ml/kg</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Diazepam</td>
<td>2.0 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Extract of <em>F.pumila</em> L</td>
<td>200mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Extract of <em>F.pumila</em> L</td>
<td>400mg/kg</td>
</tr>
</tbody>
</table>

**Table No.2: Effect of diazepam and *Ficus pumila* L. on behaviours of rats in elevated plus maze**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Number of Entries to (counts/5min)</th>
<th>Time Spend in (sec/5min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Open arm</td>
<td>Closed arm</td>
</tr>
<tr>
<td>1</td>
<td>Solvent Control</td>
<td>2.33±0.33</td>
<td>14.83 ±0.33</td>
</tr>
<tr>
<td></td>
<td>(0.5%CMC 1ml/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>2.17±0.48**</td>
<td>4.33 ±0.49**</td>
</tr>
<tr>
<td></td>
<td>(diazepam 2mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>F.pumila</em> L.</td>
<td>4.17±0.31*</td>
<td>0.17±0.47**</td>
</tr>
<tr>
<td></td>
<td>(200mg/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>F.pumila</em> L.</td>
<td>7.5±0.56**</td>
<td>6.83± 0.48**</td>
</tr>
<tr>
<td></td>
<td>(400mg/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Results are mean± S.E.M; (n = 6) Statistical comparison was performed by using ANOVA followed by Dunnet’t test. * P < 0.05, **P < 0.01, ***P < 0.001 were consider statistically significant when compared to control group)
Table No.3: Effect of diazepam and Ficus pumila L. on behavior of mice in open field

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Latency (sec/5min)</th>
<th>Time taken to enter central compartment (sec/5min)</th>
<th>Total locomotion (sec/5min)</th>
<th>Central locomotion (sec/5min)</th>
<th>Ambulation (counts/5mi)</th>
<th>Rearing (counts/5mi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle Control (0.5% CMC)</td>
<td>10.67±0.76</td>
<td>46.83±2.18</td>
<td>167.33±5.07</td>
<td>12.33±1.67</td>
<td>63.67±4.59</td>
<td>28.16±2.39</td>
</tr>
<tr>
<td>2</td>
<td>Diazepam (2mg/kg)</td>
<td>4.67±0.33**</td>
<td>23.67±2.85**</td>
<td>282.83±7.78**</td>
<td>47.67±3.38**</td>
<td>172.67±7.11**</td>
<td>8.67±0.76**</td>
</tr>
<tr>
<td>3</td>
<td>F. pumila L. 200mg/kg</td>
<td>7.33±1.17*</td>
<td>31.33±3.25**</td>
<td>224.83±13.81**</td>
<td>27.17±4.11*</td>
<td>96.83±8.60**</td>
<td>14.33±1.17**</td>
</tr>
<tr>
<td>4</td>
<td>F. pumila L. 400mg/kg</td>
<td>5.67±1.08**</td>
<td>27.67±3.07**</td>
<td>256.67±12.94**</td>
<td>38.83±5.31**</td>
<td>139.33±6.92**</td>
<td>11.33±1.43**</td>
</tr>
</tbody>
</table>

(Results are mean± S.E.M; (n = 6) Statistical comparison was performed by using ANOVA followed by Dunnet’t test. * P < 0.05, **P < 0.01, ***P < 0.001 were consider statistically significant when compared to control group).

Table No.4: Effect of diazepam and Ficus pumila L. on behaviour of mice in Y-maze model

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>Treatments</th>
<th>Number of visits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>vehicle(0.5% CMC)</td>
<td>45.67±2.11</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>diazepam(2mg/kg)</td>
<td>20.17±2.06**</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>F. pumila L. (200mg/kg)</td>
<td>37.16±2.93*</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>F. pumila L. (400mg/kg)</td>
<td>27.17±1.66**</td>
</tr>
</tbody>
</table>

(Results are mean± S.E.M; (n = 6) Statistical comparison was performed by using ANOVA followed by Dunnet’t test. * P < 0.05, **P < 0.01, ***P < 0.001 were consider statistically significant when compared to control group.)
Figure No.1: Effect of ethanolic extract of *Ficus pumila* L. on number of entries (to open/closed arms) in elevated plus-maze model

Figure No.2: Effect of ethanolic extract of *Ficus pumila* L. on time spent (in open/closed arms) in elevated plus-maze model
Figure No.3: Effect of ethanolic extract of *Ficus pumila* L. showing Ambulation and Total locomotion in Open field test

Figure No.4: Effect of ethanolic extract of *Ficus pumila* L. showing Latency, Time taken to enter central compartment, central locomotion and rearing in Open field test

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Figure No.5: Effect of ethanolic extract of *Ficus pumila* L. on mice in y-maze model

Figure No.6: Photograph of leaves of *Ficus pumila* L.
CONCLUSION
From this study, we can conclude that the ethanolic extract of *F. pumila* L. leaf extract have significant axiolytic activity in animal models. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

ACKNOWLEDGMENT
The authors are thankful to the management authorities of Nandha College of Pharmacy and Research Institute for providing necessary facilities to carry out this study.

CONFLICT OF INTEREST
We declare that we have no conflict of interest.

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