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# EVALUATION OF ANTI-ANXIETY ACTIVITY OF ETHANOLIC EXTRACT OF *RUBIA CORDIFOLIA*

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## Abstract
Anxiety is a prevalent neuropsychiatric disorder characterized by excessive worry, fear, and nervousness. The search for safer, natural alternatives to synthetic anxiolytics has led to the exploration of traditional medicinal plants. *Rubia cordifolia*, or Indian Madder, has been used in traditional medicine for various therapeutic purposes, but its anti-anxiety potential remains underexplored. This study aimed to evaluate the anxiolytic activity of the ethanolic extract of *Rubia cordifolia* and validate its traditional use. The extraction process yielded a 7.5% extract, with phytochemical analysis revealing the presence of key bioactive compounds including glycosides, flavonoids, phenolics, proteins, and diterpenes. Quantitative estimations showed a total phenol content (TPC) of 0.56 mg/100 mg and a total flavonoid content (TFC) of 0.72 mg/100 mg. Behavioral assays using the staircase and elevated plus-maze tests indicated that *Rubia cordifolia* extract significantly reduced exploratory behavior and increased time spent in open arms at a dose of 200 mg/kg, suggesting potential anxiolytic effects. In comparison, diazepam demonstrated stronger anxiolytic effects. These findings highlight *Rubia cordifolia* as a promising candidate for further research into natural anxiolytic agents, though its efficacy is currently less pronounced than that of diazepam.
**Keywords:** Anxiety, *Rubia cordifolia*, Indian Madder, ethanolic extract, anxiolytic, phytochemical analysis, phenolic content, flavonoid content, staircase test, elevated plus-maze test, diazepam.

## Introduction
Anxiety disorders are among the most prevalent mental health issues globally, significantly impacting quality of life and overall well-being. Traditional pharmacological treatments for anxiety often include benzodiazepines and selective serotonin reuptake inhibitors (SSRIs), which may cause adverse side effects and dependency issues. As a result, there is a growing interest in exploring natural alternatives for the management of anxiety.

*Rubia cordifolia*, commonly known as Indian madder or manjistha, is a perennial climbing plant belonging to the Rubiaceae family. It has been traditionally used in Ayurvedic medicine for its various therapeutic properties, including its potential neuroprotective effects (Ghosh et al., 2013). Phytochemical investigations of *R. cordifolia* have revealed the presence of various bioactive compounds such as anthraquinones, flavonoids, and tannins, which are known for their antioxidant and anti-inflammatory properties (Sharma et al., 2015). These properties suggest that *R. cordifolia* may possess anxiolytic effects.

Recent studies have begun to investigate the efficacy of herbal extracts, including *R. cordifolia*, in mitigating anxiety. For instance, a study by Gupta et al. (2016) demonstrated that extracts from *R.*

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*cordifolia* exhibited significant anxiolytic activity in animal models, potentially due to the modulation of neurotransmitter systems. However, the precise mechanisms and therapeutic potential of *R. cordifolia* in anxiety management remain underexplored.

This study aims to evaluate the anti-anxiety activity of the ethanolic extract of *Rubia cordifolia* using established behavioral models in animals. By assessing the anxiolytic potential of this extract, we hope to contribute to the growing body of evidence supporting the use of herbal remedies in anxiety treatment.

## Material and Methods

### Material

The study utilized a range of chemicals and reagents sourced from reputable suppliers to ensure accuracy and reliability in the experimental procedures. Key materials included Potassium Mercuric Iodide**,** Iodine, and Potassium Iodide from Loba Chemie Pvt. Ltd. for analytical tests, along with Sodium Nitroprusside and Sodium Hydroxide for chemical reactions. Gelatin and Folin-Ciocalteu reagent were employed for biochemical assays, while solvents such as Methanol**,** Ethanol, and Chloroform from Qualigens Fine Chemicals facilitated extraction and analysis. Other essential reagents included **F**erric Chloride**,** Lead Acetate, and Copper Acetate sourced from Thomas Baker and S. D. Fine Chem. Ltd. These materials collectively supported the evaluation of the anti-anxiety activity of the ethanolic extract of Rubia cordifolia.

### Methods

#### Collection of plant material

The plants have been selected on its availability and folk use of the plant. Every parts of the plant like bark, leaves, flowers, roots, fruits and seeds may contain active secondary metabolites. Fresh and healthy plant materials, free from diseases of *Rubia cordifolia* were collected from ruler area of Bhopal (M.P.) in the month of March, 2024.

#### Extraction procedure

Extraction is an essential step in phytochemical processing for the finding of bioactive secondary metabolite from plant materials. For the standardization of herbal products, selection of a suitable extraction technique is also important. Extraction is used in the removal of desirable soluble constituents, exclusion those not required with the help of the selected solvents. The collected plant materials were thoroughly washed in tap water and rinsed in distilled water. The cleaned, healthy collected plant samples were cut into small pieces and dried under shade for 3 to 4 weeks. Following procedure will be adopted for the preparation of extract from the shade dried material (Ansari, 2001).

#### Defatting of plant material

60 gram of *Rubia cordifolia* shade dried plant material were coarsely powdered and subjected to extraction with petroleum ether in a maceration method. The extraction was continued till the defatting of the material had taken place.

#### Extraction by maceration process

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http://magellanes.com/

Defatted plant materials of *Rubia cordifolia* were extracted with ethanol solvent by maceration method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts (Mukherjee, 2007).

**Determination of percentage yield**

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples and it was defined as quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{Weight\ of\ Extract}{Weight\ of\ Powder\ drug\ taken} x\ 100$$

**Phytochemical screening**

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods (Khandelwal, 2005).

**Quantitative estimation of bioactive compounds**

**Total phenolic content estimation**

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Parkhe and Bharti, 2019).

**Total flavonoids content estimation**

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm (Parkhe and Bharti, 2019).

**Anti-anxiety activity**

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http://magellanes.com/

The anti-anxiety activity was evaluated using staircase test and elevated plus maize test.

**Dosing and grouping of animals**

Swiss albino mice were taken and divided into four groups, each group comprised of 6 animals. The two doses of ethanolic extract of *Rubia cordifolia* (100 and 200mg/kg) were administered orally, the standard group was treated with diazepam (4mg/kg) intraperitoneally and control group received Tween 80 (2% w/v) orally.

**Table 6.3: Dosing and grouping of animals**

| S. No | Groups | Dose |
|---|---|---|
| Group-II | Control | Vehicle 6 ml/kg, p.o |
| Group-III | Treated with ethanolic extract of *Rubia cordifolia* | 100mg/kg, p.o |
| Group-IV | Treated with ethanolic extract of *Rubia cordifolia* | 200mg/kg, p.o |
| Group-V | Treated with (Std) Diazepam | 4mg/kg, i.p |

**Staircase test**

Staircase consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The internal height of the walls is constant along whole length of the staircase. The animals were placed on the floor of the box with its back to the staircase. The number of steps climbed and the number of rears were counted over a 3 min period. A step was considered to be climbed only if the mouse had placed all four paws on the step. In order to simplify the observation, the numbers of steps descended were not taken into account. After each step the box was cleaned in order to eliminate any olfactory cues, which might modify the behavior of the next animal (Rakotonirina *et al.,* 2001).

**Elevated plus maze**

The apparatus consist of two open arms (5 × 10 cm) and two closed arms (5 × 10 × l5 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. The animal was placed at the center of the maze, facing one of the closed arms. During 5 min test period the following measures are taken:

• The number of entries into open arms

• The number of entries into closed arms

• Time spent in the open arms

Arm entry was counted when the animal had placed all of its four paws on it. The procedure was conducted in a sound attenuated room, with observations made from an adjacent room (Nishikava *et al.,* 2004).

**Statistical analysis**

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http://magellanes.com/

Results were expressed as Mean ± SEM the differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Dennett's test and were considered statistically significant when $P<0.05$.

**Results and Discussion**

The evaluation of the ethanolic extract of *Rubia cordifolia* revealed a percentage yield of 7.5%, indicating a moderately efficient extraction process. Phytochemical screening identified the presence of key constituents, including glycosides, flavonoids, phenolics, and proteins, while alkaloids and saponins were absent. These findings align with previous studies highlighting the phytochemical richness of *Rubia cordifolia*, which may contribute to its medicinal properties.

The total phenolic content was estimated at 0.56 mg/100 mg, while total flavonoids were found to be 0.72 mg/100 mg. Both phenolic compounds and flavonoids are known for their antioxidant properties and potential role in anxiety modulation, which supports the rationale for evaluating the extract's anxiolytic effects.

In the staircase test, the ethanolic extract at doses of 100 mg/kg and 200 mg/kg showed a significant decrease in the number of climbs and rearings compared to the control group. This suggests a potential anxiolytic effect, which was further corroborated by the elevated plus-maze test. Here, a reduction in entries into the closed arms and increased time spent in the open arms at the higher dose indicates reduced anxiety-like behavior. The results were comparable to those obtained with diazepam, a standard anxiolytic, further validating the efficacy of the extract.

**Table 1: Results of percentage yield of extract of *Rubia cordifolia***

| S. No. | Ethanolic extract | Percentage yield (w/w) |
|---|---|---|
| 1 | *Rubia cordifolia* | 7.5% |

**Table 2: Result of phytochemical screening of ethanolic extract of *Rubia cordifolia***

| S. No. | Constituents | Ethanolic extract |
|---|---|---|
| 1. | **Alkaloids** | |
| | A) Wagner's Test: | -Ve |
| | B) Hager's Test: | -Ve |
| 2. | **Glycosides** | |
| | A) Legal's Test: | +Ve |
| 3. | **Flavonoids** | |
| | A) Lead acetate Test: | +Ve |
| | B) Alkaline reagent Test: | +Ve |
| 4. | **Saponins** | |
| | A) Froth Test: | -Ve |
| 5. | **Phenolics** | |
| | A) Ferric chloride Test: | +Ve |
| 6. | **Proteins** | |

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| | | |
|---|---|---|
| | A) Xanthoproteic Test: | +Ve |
| 7. | **Carbohydrate**<br>A) Fehling's Test: | -Ve |
| 8. | **Diterpenes**<br>A) Copper acetate Test: | +Ve |

**Table 3: Estimation of total phenolic and flavonoids content of *Rubia cordifolia***

| S. No. | Ethanolic extract | Total phenol content | Total flavonoids content |
|---|---|---|---|
| 1. | *Rubia cordifolia* | 0.56 mg/100mg | 0.72 mg/100mg |

**Table 4: Effect of ethanolic extract of *Rubia cordifolia* and diazepam in stair case test and elevated plus-maze test**

| Groups | Stair case test | |
|---|---|---|
| | No. of climbing in 3 min | No. of rearing in 3 min |
| Control (Vehicle 6 ml/kg, p.o) | 19.25±1.37 | 8.50±0.30 |
| Ethanolic extract of *Rubia cordifolia* (100 mg/kg, p.o) | 12.50±0.80 | 7.25±0.35 |
| Ethanolic extract of *Rubia cordifolia* (200 mg/kg, p.o) | 7.00±0.60** | 5.85±0.55** |
| Diazepam (4 mg/kg, i.p) | 5.10±0.67** | 4.10±0.50** |

All values are Mean ± SEM, n = 6, *P when compared with control.

**Table 5: Effect of ethanolic extract of *Rubia cordifolia* and diazepam in stair case test and elevated plus-maze test**

| Groups | Elevated plus maze test | | |
|---|---|---|---|
| | No. of entry into | | Time spent in open arms |
| | Closed arms | Open arms | |
| Control (Vehicle 6 ml/kg, p.o) | 12.25±1.30 | 8.50±1.85 | 92.36±6.15 |
| ethanolic extract of *Rubia cordifolia* (100 mg/kg, p.o) | 8.90±1.65 | 5.82±0.95 | 105.12±8.95 |
| ethanolic extract of *Rubia cordifolia* (200 mg/kg, p.o) | 6.68±1.15** | 5.92±1.65 | 150.32±7.56* |
| Diazepam (4 mg/kg, i.p) | 6.38±0.97** | 3.95±1.36* | 195.65± 12.15** |

All values are Mean ± SEM, n = 6, *P when compared with control.

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## Conclusion

The ethanolic extract of *Rubia cordifolia* demonstrates promising anti-anxiety activity, as evidenced by behavioral assessments in the staircase and elevated plus-maze tests. The significant reduction in anxiety-like behaviors at higher doses supports the therapeutic potential of this extract, likely attributable to its phytochemical constituents such as glycosides, flavonoids, and phenolics. The findings suggest that *Rubia cordifolia* may serve as a valuable natural alternative for anxiety management. Further studies are recommended to explore its mechanisms of action and to evaluate its efficacy in clinical settings.

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