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# EVALUATION OF ANXIOLYTIC EFFECT OF FLOWER OF *COURAPITA GUINIASIS*

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***Abstract:***
Couroupita guianensis Aubl. A member of the Lecythidaceae family, often referred to as cannonball, thrives in the tropical regions of India, South America, and the Caribbean. The plant is a significant source of triterpenoids associated with anxiolytic action. The current study aims to assess the possible anxiolytic effects of the methanolic extract of Couroupita guianensis (CGFM) flowers in mice. Swiss albino male mice received oral doses of 100 and 200 mg/kg of both aqueous and methanolic extracts of CG for a duration of 15 days. The Elevated plus Maze (EPM), Forced Swim Test (FST), and Open Field Test (OFT) models were used to assess anxiolytic activity. The aqueous and methanolic extracts of CG at a dosage of 200 mg/kg exhibited considerable anxiolytic efficacy compared to the vehicle control in the EPM, FST, and OFT models in mice. This is the first research documenting the anxiolytic potential of CG flower extracts. Additional research is required to isolate the active component to validate mechanism-based action.
***Keywords:*** Evaluation, Anxiolytic effect, flower and *Courapita guiniasis*.

## 1 INTRODUCTION

Research in the field of psychopharmacology has focused a significant amount of attention on anxiety, which affects one-eighth of the world's population. Studying plant extracts for their anxiolytic effects in animal models is becoming more important as scientific research continues to advance and new drugs are produced. Although they are linked to drug dependence, sleepiness, tremors, and forgetfulness, benzodiazepines constitute the cornerstone of pharmacological treatment for anxiety disorders. This is despite the fact that they are associated with certain side effects. For the time being, benzodiazepines make up the vast bulk of the drugs that are prescribed for anxiety disorders. This is because benzodiazepines have a number of negative effects, some of which include dependence, psychomotor impairment, and the enhancement of other central depressants. These side effects limit the therapeutic usefulness of the drug. It is of the utmost importance that new anxiolytic medicines that do not have the negative effects of benzodiazepines be developed for the treatment of anxiety disorders [1-3].

The study on medicinal plants has made significant progress all over the globe, showing the pharmacological activity of a number of different plant species across a broad range of animal models. This research is being conducted in an effort to find innovative treatment choices for neurological and behavioral diseases. The discovery of innovative anxiolytic drugs that have superior safety profiles, shorter half-lives, and larger therapeutic windows has garnered a greater amount of attention from researchers in recent years. The unusual floral structure of the *Couroupita guianensis Aubl.* (Lecythidaceae) tree, which is a tropical tree that is endemic to the Amazon rainforest and deciduous, is one of its most notable characteristics. Extensive research has been conducted on a variety of plant qualities, including their depressive, antibacterial, larvicidal, and immunomodulatory effects.

Additionally, inflammation, tumors, and pain throughout the stomach have all been relieved as a result of its lengthy history of use [4].

There is a high incidence of this phenomenon, particularly in tropical America and the West Indies. Alkaloids, tannins, and terpenes are elements that are considered to be among the most significant components of plants. Previous research has successfully extracted a number of chemicals from different plant components, such as flowers, seeds, fruits, and leaves. These compounds include α, β-amyrin, stigmasterol, β-sitosterol, campesterol, linoleic acid, eugenol, linalool, farnesol, nerol, tryptanthrin, indigo, indirubin, isatin, and carotenoids, among others. On the other hand, there is a dearth of published research on the underlying reasons, and there is also a dearth of substantial scientific examinations that demonstrate the antidepressant qualities of the substance. Using a battery of pharmacological screening tests, the purpose of this research was to identify whether or not *Couroupita guianensis* had any feelings of anxiety-reducing properties. Eugenol, linalool, and (E, E)-farnesol were the molecular components of flowers that received the greatest attention due to their extensive research. In addition, the plant contains significant amounts of triterpenoids, which have recently been associated with the provision of anxiolytic effects. In an earlier investigation that included a CG root methanolic extract in our laboratory, it was shown that the extract had anxiolytic effects. This research is the first of its kind to evaluate the effectiveness of *C. guianensis* flower extract as an anxiolytic against anxiety at dosages of 100 and 200 mg/kg. For the purpose of determining whether or not the anxiolytic was effective, animal tests were conducted using the Open Field Test (OFT), the Forced Swim Test (FST), and the Elevated Plus Maze (EPM respectively [5, 6].

## 2 MATERIAL AND METHODS

### 2.1 Collection and Authentication of Plant material

Courapita guianensis was examined by taxonomists from the Botanical Survey of India in the Empress Botanical Garden in Pune Cantonment, Pune, Maharashtra, over two distinct periods: from February to June and from September to December.

Figure 1 Flower of *Cannon ball*

### 2.2 Extraction of Plant Extract

We analyzed an authentic specimen of *Courapita guianensi*s. A designated quantity of plant material was macerated in fifty milliliters of distilled water for one hour for each formulation. The amalgamation was simmered for twenty minutes. Employ a Whatman paper filter to strain the mixture after it has cooled. In a supplementary study, *Courapita guianensis* powder was subjected to solvent extraction using several solvents, such as water and alcohol [7].

### 2.3 Purification of Plant Extract

We analyzed solid-liquid extracts obtained from methanol, acetone, and n-hexane using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HP-TLC). Recrystallization in a solvent devoid of impurities may result via column chromatography, which enhances the polarity of the solvent system. To assess, among other factors, the amount and integrity of natural chemicals, including flavonoids and triterpenoids. Establishing chromatographic settings is crucial for achieving separation goals. Flavonoids have been separated and purified from hydroalcoholic and ethyl acetate extracts to generate various derivatives, which are extracted using a 1:1 hydroethanolic mixture for flavonoid separation. Isolated flavonoids were analyzed using thin-layer chromatography (TLC), ultraviolet (UV) spectroscopy, and thermo fluorescence (IR) techniques. The coarsely ground Courapita guianensis plant material underwent sequential solvent extraction

using a Soxhlet system, utilizing various solvents arranged by ascending polarity. I shall begin with ethyl acetate, methanol, chloroform, and petroleum ether. The water extract was produced by the maceration process [8]. Upon completion of the extraction, the solvent was distilled, the residue concentrated, and the mixture let to dry. Following each solvent extraction, the marc was let to thoroughly air dry prior to the next extraction. The vacuum-dried extracts included several phytoconstituents, as ascertained by standard chemical laboratory methods. Floral plants contain several chemicals, including geraniol, triterpenoids, terpenes, saponins, steroids, carbohydrates, polyphenols, and tannins [9].

### 2.4 Animals

Six male albino mice (Swiss, weighing 22–25 g) were housed in a controlled environment with conventional laboratory lighting, humidity, and temperature. Unless stated otherwise, all animals were provided with full access to food and water. They were forbidden from ingesting food or drinks for twelve hours before the drug was administered. Each cohort had six animals in total. All investigations were performed during daytime hours. This study adhered to the rules set out by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in New Delhi, India, and received approval from the Institutional Animal Ethical Committee [10].

### 2.5 Acute Toxicity Study

The OECD-423 guidelines were used to perform the acute toxicological study. Swiss albino mice, including both sexes, were used in the studies. The animals were had unrestricted access to water during their four-hour fast. Six groups were formed, each including six animals. Mice received oral administration of CGM dissolved in distilled water at doses of 500, 750, 1000, 1250, 1500, and 2000 mg/kg of body weight. Mice were monitored for signs of toxicity and death during the first 24 hours and then on a daily basis for the next 14 days [11, 12].

### 2.6 Anxiolytic Activity Evaluation

#### 2.6.1 Elevated Plus Maze (EPM)

Rats participated in the Elevated plus Maze (EPM) test, which evaluates hesitant behavior, by allocating time to both open and enclosed arms. Rats like dim settings, akin to a retracted limb; hence, they will inhabit such areas when alone. The rat was situated in the midpoint of an elevated plus maze for this experiment. Two open and two closed limbs protruded from the central platform of the apparatus, positioned 50 cm above the ground. The study took place throughout the light portion of the light/dark cycle, namely from 10:00 AM to 1:00 PM. The item was preserved in an environment with a steady background noise level of 50 db. We gathered data on the animal's overall length of stay and the frequency of its entries into each arm type. Animals with elevated anxiety levels have a reduced rate of admission and a limited duration of open-arm activity [13].

#### 2.6.2 Open Field Test (OFT)

We computed the aggregate number of squares crossed by all four limbs throughout a duration of six minutes. To eliminate any signs of animal activity, the device was sanitized with a 10% ethanol solution between each test [14].

#### 2.6.3 Forced swim test (FST)

The procedure described in this publication included the solitary swimming of rats in a cylindrical open container filled with 19 cm of water, maintained at a temperature of 25 ± 1 °C. The period of immobility was then quantified. Upon the cessation of rodent movements and the animals' peaceful repose in the water, gently oscillating their heads laterally, we deemed them to be still. The test treatment's anxiolytic efficacy is shown by a decrease in immobility length [15].

#### 2.6.4 Brain model

After completion of all behavioral evaluation, animals were sacrificed by $CO_2$ euthanasia, and upon the conclusion of all behavioral assessments, the animals were killed using CO2. Thereafter, the brain was excised, homogenized, centrifuged, and immersed in a cooled phosphate buffer solution. An ELISA reagent was used to quantify the quantity of GABA in the supernatant. Extracts from the Courapiata guiniesis flower, mediated by gamma-amino butyric acid (GABA), provide anxiolytic effects, as shown by in vivo studies [16].

**Table 1Grouping and treatment for anxiolytic activity evaluation of *Courapita guiniasis***

| Groups | Treatment | Dose (mg/kg), route | No. animals |
|---|---|---|---|
| Group I | Normal control, Normal saline solution | 10 mL/kg, oral | 06 |
| Group II | Anxiety control (stress induced) | 10 mL/kg, oral | 06 |
| Group III | Standard control (diazepam) | 5 mg/kg, i.p | 06 |
| Group IV | *Courapita guiniasis*extract dose 1 | 100 mg/kg | 06 |
| Group V | *Courapita guiniasis* extract dose 2 | 200 mg/kg | 06 |
| Total animals | | | 30 |

## 3 RESULT AND DISCUSSION

### 3.1 Phytochemical Screening

The first phytochemical analysis of Couroupita guianensis (CGM) identified the presence of triterpenoids, flavonoids, alkaloids, and glycosides.

### 3.2 Acute Toxicity Test

No fatalities were recorded over the 72-hour duration of the acute toxicity trial at the administered levels. At these dosages, the animals exhibited no classic signs indicative of toxicity, such as convulsions, ataxia, diarrhea, or increased diuresis. The median lethal dosage (LD50) was established to exceed the tested amount of 2.0 g/kg.

### 3.3 Anxiolytic Activity Evaluation

#### 3.3.1 Elevated Plus Maze (EPM)

**Table 4 Effects of *Courapita guiniasis* extract on time spent in open arm and Entries in open arm by elevated plus maze in acute restraint stress induced anxiety like behavior in rats**

| **Groups and Treatment** | **Time spent in open arm (seconds; Mean±SD)** | **Entries in open arm (%)** |
|---|---|---|
| Normal Control | 73.67±17.2 | 34.67±10.23 |
| Restraint stress control | 36.00±12.44## | 17.17±6.23## |
| Standard group (Diazepam 2 mg/kg) | 98.00±13.55** | 29.33±7.92* |
| Courapita guiniasis 100 mg/kg | 106.00±31.92** | 16.50±6.43 |
| Courapita guiniasis extract 200 mg/kg | 127.67±12.37** | 34.67±8.82** |
| Data was analyzed by one way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group. | | |

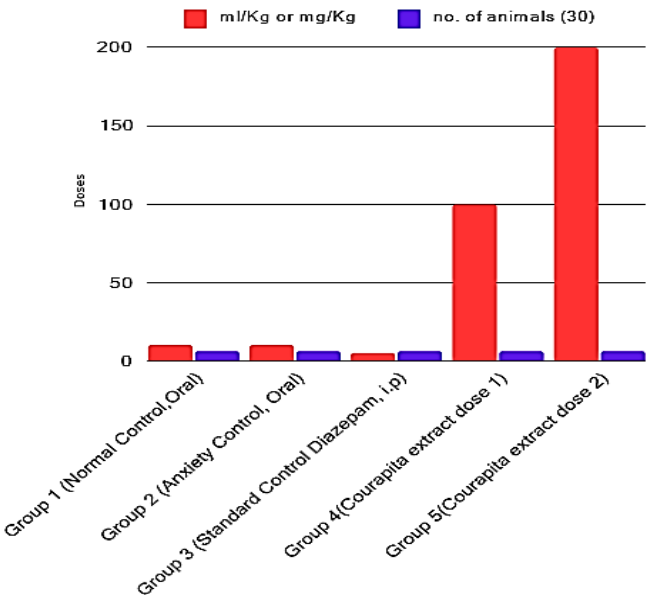


**Figure 2Effects of *Courapita guiniasis* extract on time spent in open arm and Entries in open arm by elevated plus maze in acute restraint stress induced anxiety like behavior in rats.**

Data was analyzed by one-way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group.

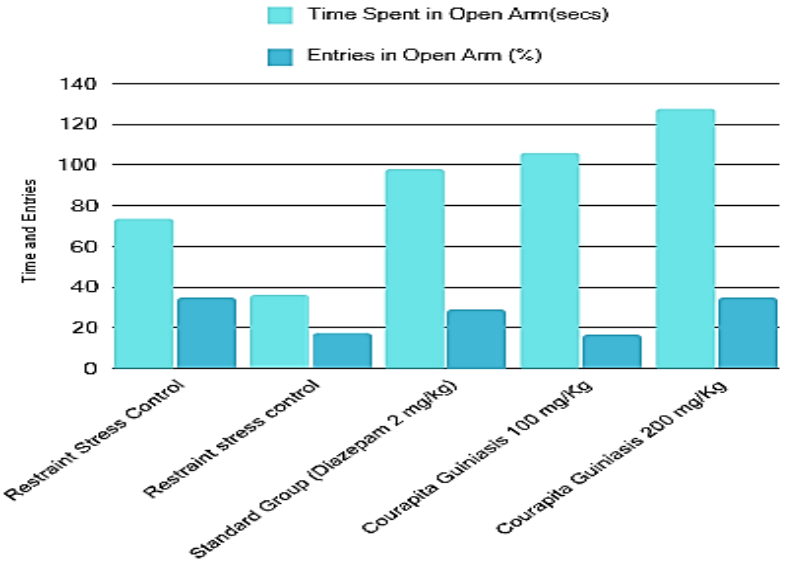


**Figure 3Effects of *Courapita guiniasis* extract on Entries in open arm by elevated plus maze in acute restraint stress induced anxiety like behavior in rats.**

Data was analyzed by one-way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group.

### 3.3.2 Open Field Test (OFT)

**Table 5Effects of *Courapita guiniasis* extract on number of crossings by open filed test in acute restraint stress induced anxiety like behavior in rats**

| **Groups and Treatment** | **number of crossings (numbers; Mean±SD)** |
|---|---|
| Normal Control | 139.67±26.58 |
| Restraint stress control | 125.50±19.84# |
| Standard group(Diazepam 2 mg/kg) | 95.17±10.48* |
| Courapita guiniasis 100 mg/kg | 132.83±28.11 |
| Courapita guiniasis extract 200 mg/kg | 115.63±29.43 |
| Data was analyzed by one way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group. | |

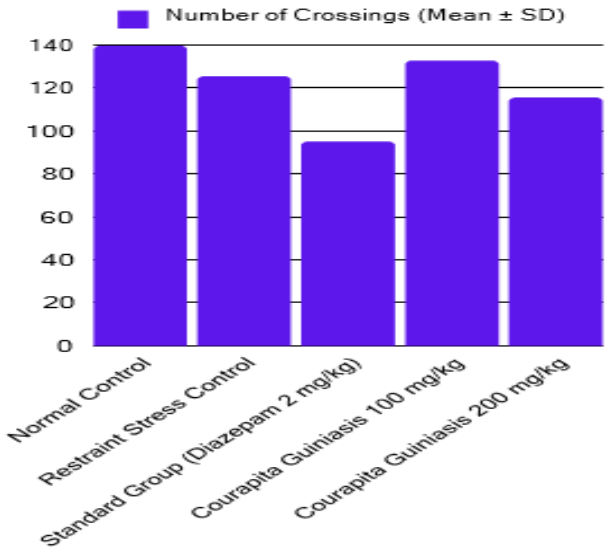


**Figure 4Effects of *Courapita guiniasis* extract on number of crossings by open filed test in acute restraint stress induced anxiety like behavior in rats.**

Data was analyzed by one-way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group.

### 3.3.3 Forced swim test (FST)

**Table 6Effects of Courapita guiniasis extract on immobility time by forced swim test in acute restraint stress induced anxiety like behavior in rats**

| **Groups and Treatment** | **Immobility time (second; Mean±SD)** |
|---|---|
| Normal Control | 114.33±17.22 |
| Restraint stress control | 205.83±20.32## |
| Standard group(Diazepam 2 mg/kg) | 121.17±23.23** |
| Courapita guiniasis 100 mg/kg | 134.73±10.34** |
| Courapita guiniasis extract 200 mg/kg | 134.50±27.77** |
| Data was analyzed by one way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group. | |

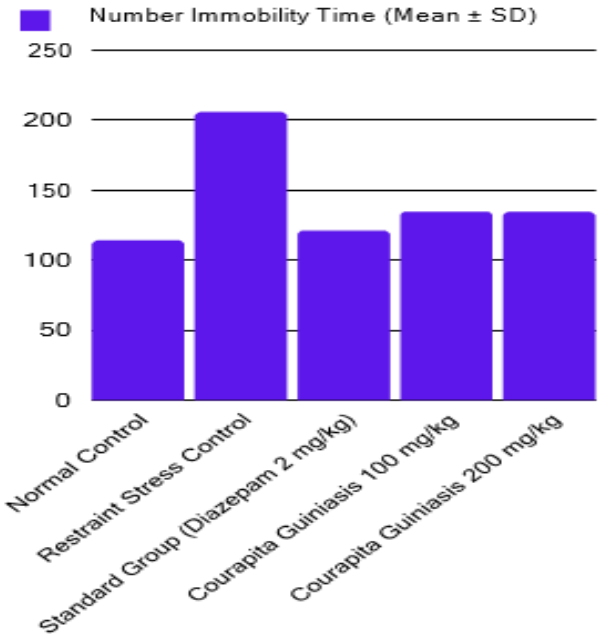


**Figure 5Effects of *Courapita guiniasis* extract on immobility time by forced swim test in acute restraint stress induced anxiety like behavior in rats.**

Data was analyzed by one-way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group.

### 3.3.4 Brain model

**Table 7Effects of *Courapita guiniasis* extract on GABA levels in acute restraint stress induced anxiety like behavior in rats**

| **Groups and Treatment** | **GABA levels (pg/mg of tissue) (Mean±SD)** |
|---|---|
| Normal Control | 44.67±10.38 |
| Restraint stress control | 104.00±21.84# |
| Standard group(Diazepam 2 mg/kg) | 156.50±38.01** |
| Courapita guiniasis 100 mg/kg | 132.84±28.89 |
| Courapita guiniasis extract 200 mg/kg | 161.00±20.63* |
| Data was analyzed by one way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group. | |

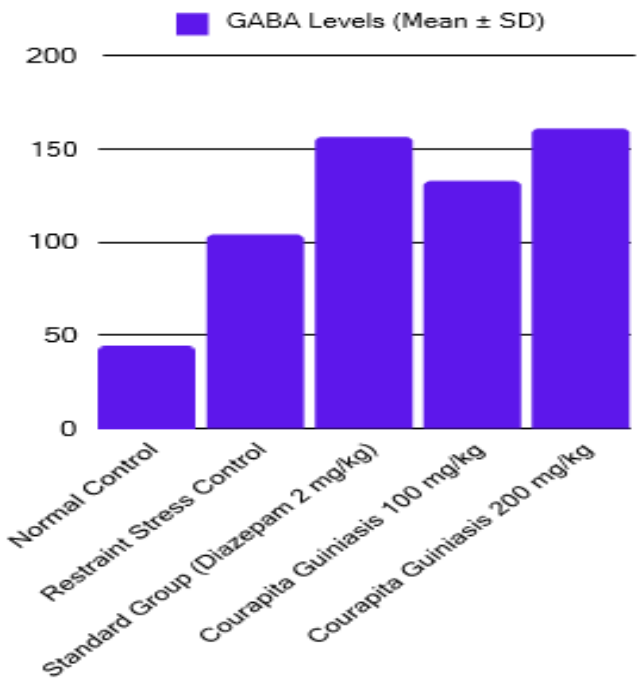


**Figure 6Effects of *Courapita guiniasis* extract on GABA levels in acute restraint stress induced anxiety like behavior in rats.**

Data was analyzed by one-way ANOVA followed by Bonferroni post hoc test, ##p<0.001 when compared to normal control group, ** p<0.001 compared to Acute restrain stress group.

## 4 SUMMARY AND CONCLUSION

Medicinal herbs have long been the go-to remedy for a wide range of illnesses. Anticancer, antiulcer, antinociceptive, antifungal, antifertility, antimicrobial, antioxidant, and anxiolytic properties are shown by the medicinal plant known as Couroupita guianensis. Finding, selecting, and using just the plant components that exhibit maximum therapeutic efficacy is crucial in light of the ongoing challenges in standardizing plant-based medications [17, 18]. Both the water and alcohol extracts of *C. guianensis* have shown to be effective in reducing anxiety. In addition, our findings point to the plant extracts' triterpene-rich fractions as a possible explanation for the anxiolytic effect. This could be because GABAergic transmission, which is involved in the physical expression of anxiety, is enhanced when certain central recognition sites linked to GABA receptors are engaged. In addition, research into the active ingredient accountable for the anxiolytic activity must be conducted [19, 20].

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