



Archives available at journals.mriindia.com

International Journal of Recent Advances in Engineering and Technology

ISSN: 2347-2812

Volume 14 Issue 1s, 2025

Anxiolytic Effect of Eugenol In *Courapita Guiniasis* Flower Along With Hptlc Analysis

Aparna Deodhe^{*1}, Dr. Rakesh Kumar Jat²

¹Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, University in Jhunjhunu, Rajasthan, India.

²Assistant professor, Shri Jagdishprasad Jhabarmal Tibrewala University, University in Jhunjhunu, Rajasthan, India.

Peer Review Information

Submission: 19 Jan 2025

Revision: 21 Feb 2025

Acceptance: 25 March 2025

Keywords

HPTLC

Eugenol

Courapita Guiniasis

Anxiolytic Effect

Abstract

The objective of this study was to isolate and purify eugenol from the methanolic extract of the Cannonball Flower (*Couroupita guianensis*). The presence of eugenol in the extract was confirmed using High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting. Standard eugenol was employed as a reference marker for comparison. The study also aimed to evaluate the potential anxiolytic effects of methanolic extracts of Cannonball Flower in rats. Male Swiss albino rats were administered 100 and 200 mg/kg of aqueous and methanolic extracts of Cannonball Flower (CG) orally for 15 consecutive days. The anxiolytic activity was assessed using the Open Field Test (OFT), Forced Swim Test (FST), and Elevated Plus Maze (EPM) models. Eugenol was detected in the Cannonball Flower extract at concentrations of 4, 6, and 8 µg/ml, showing distinct blue bands (T1, T2, and T3) in the HPTLC chromatogram at 366 nm. Both the standard eugenol and the Cannonball Flower extract were analyzed at the same concentrations, confirming the presence of eugenol in all three levels. The results indicated that, compared to the vehicle control, both the aqueous and methanolic extracts of CG at a dose of 200 mg/kg significantly exhibited anxiolytic effects in the EPM, FST, and OFT models. This is the first exploration of the anxiolytic properties of CG flower extracts.

INTRODUCTION

Studies in the field of psychopharmacology have focused a significant amount of attention on anxiety, which affects one in eight persons throughout the world. A heightened interest in the anxiolytic effects of botanical extracts in animal research has been sparked as a result of the fast development of scientific inquiry and the production of novel medications. In spite of the potential for dependence, ataxia, amnesia, drowsiness, and relaxing of the muscles, benzodiazepines continue to be the most

effective pharmaceutical intervention for anxiety disorders. By far the most common kind of pharmacological treatment for anxiety disorders is the use of benzodiazepines. In spite of this, the therapeutic use of benzodiazepines is restricted due to the bad effects that they have. These adverse effects include psychomotor impairment, the potentiation of other central depressants, and the possibility of dependence. The development of novel medications that include anxiolytic properties and do not include the drawbacks that are associated with

benzodiazepines might prove to be beneficial for the treatment of disorders that are associated with anxiety (1-3).

As a result of research on medicinal plants, the quest for novel therapeutic agents for neurological and behavioral issues has been continually driven throughout the globe. This research has shown the pharmacological efficacy of several plant species across a broad variety of animal models. For the most part, researchers have focused their attention on developing new anxiolytic compounds that have a rapid onset of action, fewer adverse effects, and a wider therapeutic index. The *Couroupita guianensis* tree, which belongs to the family Lecythidaceae, is a tropical tree that is deciduous and native to the Amazon rainforest. It is identified by the blooms that it bears. Several studies have shown that plants possess a wide range of medicinal properties, such as immunomodulatory, larvicidal, antibacterial, and antidepressant effects. In addition to this, it has been used for a considerable amount of time in order to alleviate pain in the gastrointestinal tract, tumour, and inflammation (4, 5).

It may be found in large numbers over the tropical regions of America and the West Indies. The plants contain a number of phytoconstituents, the most important of which being triterpenes, tannins, and alkaloids. There are a multitude of compounds that have been extracted from flowers, seeds, fruits, and leaves in previous research. These chemicals include α , β -amyrin, stigmasterol, β -sitosterol, campesterol, linoleic acid, eugenol, linalool, farnesol, nerol, tryptanthrin, indigo, indirubin, isatin, and carotenoids, among others. Nevertheless, there has not been any exhaustive scientific study conducted to substantiate its efficacy as an antidepressant, nor has there been any investigation carried out to determine the underlying rationale for its effectiveness. Several different pharmacological screening methods were used in order to accomplish the objective of this research, which was to examine the anxiolytic characteristics of *Couroupita guianensis*. Eugenol, linalool, and (E, E)-farnesol that were found in the flowers were the primary chemical components that were found in the flowers. An additional benefit of the plant is that it is a significant source of triterpenoids, which have been associated with the reduction of anxiety. The results of a previous investigation conducted in our laboratory indicate that the methanolic extract of CG roots may have the potential to have anxiolytic effects. For the first time, we performed an investigation into the efficacy of *C. guianensis* flower extract as an anxiolytic agent by administering doses of 100 and 200 mg/kg. The Elevated plus Maze (EPM), the Forced Swim Test (FST), and the Open Field Test (OFT) were the animal models that were

used in order to test the anxiolytic impact of the medicine (6, 7).

MATERIAL AND METHODS 1. Collection and Authentication of Plant material

Flowers of *Courapita guianensis* were gathered in the Empress Botanical Garden in Pune Cantonment, Maharashtra, from February to June and again from September to December. A taxonomist from the Botanical Survey of India conducted the authentication.



Figure 1 Flower of Cannon ball

2. Extraction of Plant Extract

A specimen of *Courapita guianensis* was verified. A specified amount of plant powder was macerated in 50 milliliters of distilled water for one hour for each formulation. The amalgamation was cooked for twenty minutes. Employ Whatman filter paper for filtration subsequent to cooling. Alcohol, water, and other mixtures may be used to extract the solvent from powdered *Courapita guianensis* in a distinct experiment (8).

3. Purification of Plant Extract

Methanol, acetone, and n-hexane were used to produce solid-liquid extracts, and highPerformance Thin Layer Chromatography (HP-TLC) was utilised for the analysis of the findings (9).

a. HPTLC Analysis

An HPTLC investigation was conducted on the methanolic extract of *cannonball* flowers. Highperformance thin-layer chromatography was performed using silica gel 60F 254 (10 cm $\times$ 10 cm; 0.25 mm layer thickness; Merck). The *Cannon Ball* Flower Extract was obtained by diluting 10 mg of the extract in methanol to a final volume of 10 ml in a volumetric container. Each sample was subjected to filtration using a 0.45 micron syringe filter at a concentration of 1000 μ g/ml before being analyzed by HPTLC at concentrations of 4, 6, and 8 μ g/ml (10). Three separate quantities of each extract were discerned on a silica gel 60F254 TLC plate. The plate was air-dried and then produced in a CAMAG twin-trough glass chamber, which was immersed in mobile phase vapour for 20

minutes. The solvent system consisted of hexane, chloroform, and methanol at a volume ratio of

4:4:2. The plate was analyzed using WinCATS 4 software with a CAMAG Scanner 3 (CAMAG, Switzerland) at wavelengths of 254 and 366 nm, after a two-minute development and drying period at 65°C. A stock sample of cannon ball flower extract was prepared at a concentration of 1000 µg/ml, whereas a stock standard of eugenol was prepared at 200 µg/ml (11).

b. Anxiolytic Activity Evaluation

The anxiolytic effects of aqueous and methanolic floral extracts of *Couroupita guianensis* include the open field test (OFT), light-dark (LD) test, and elevated plus maze (EPM) models were assessed. The findings demonstrate that 500 mg/kg of *Couroupita guianensis* extract (aqueous and methanolic) had an anxiolytic effect in mice, correlated with vehicle administration in LD, EPM, and open field assessments (12, 13).

RESULT AND DISCUSSION 1. Phytochemical Screening

Couroupita guianensis (CGM) yielded triterpenoids, flavonoids, alkaloids, and glycosides after first phytochemical analysis. The concentration of flavonoids in various plant components was quantitatively evaluated using ferric chloride and sodium hydroxide tests. Only the fruit epidermis, floral anthers, and floral petals demonstrate flavonoid concentration in the NaOH test; leaves showed no positive findings or flavonoid content. Furthermore, ferric chloride analysis revealed the abundant presence of flavonoids inside the plant. *C. guianensis* was originally thought to possess several active constituents, including an abundance of flavonoids.

2. HPTLC Analysis

The HPTLC fingerprinting profile is essential for the precise identification of medicinal plants and, therefore, for the standardization of herbal medicine. Subsequent analysis was performed using HPTLC on four separate extracts, excluding the pet ether extract, in alignment with the findings of the chemical test.

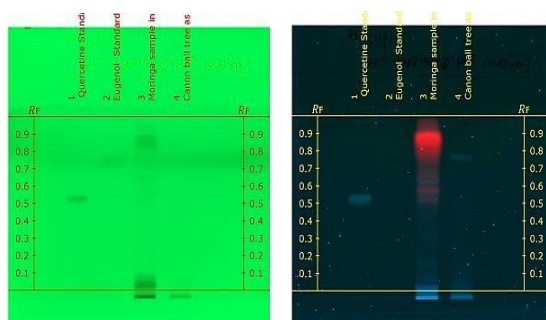


Figure 2 TLC plates scanned at 254 and 366 nm for *C. guianensis* flower extracts Track1 (Quercetine Standard 1 mg / ml), Track2 (Eugenol Standard 1 mg / ml), Track3 (Moringa sample in 100% Methanol) and Track4 (Canon ball tree as such sample)

A variety of solvent solutions were evaluated for cannabis flower extracts; however, Toluene: Ethyl acetate yielded an adequate resolution. The ratio of formic acid to methanol in floral extracts is 5.5:3:1:0.5. The HPTLC chromatogram of several cannonball flower extracts is shown in Figures 2 at 254 and 366 nm. In our investigation, the phenolic compound eugenol was detected in the extract of cannabis flowers using HPTLC fingerprinting. The HPTLC examination performed at both 254 and 366 nm confirmed this. The standard (Eugenol) and cannonball flower extract were fingerprinted using HPTLC at doses of 4, 6, and 8 µg/ml. Eugenol was identified in the Cannon Ball Flower extract across all three doses.

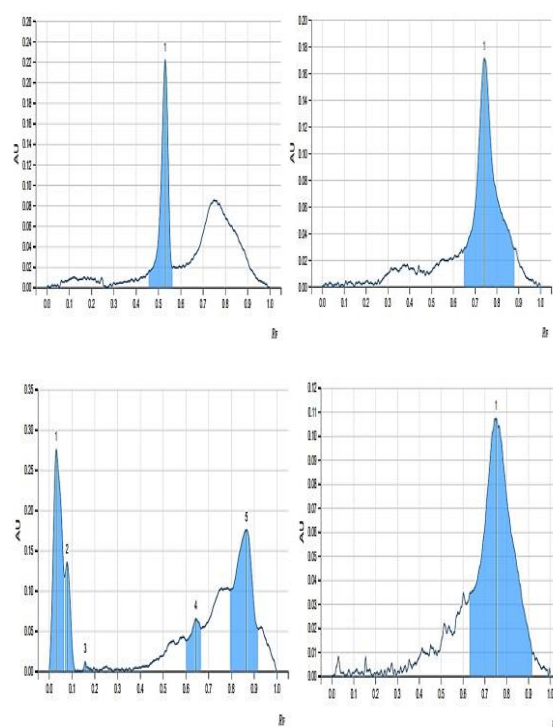


Figure 3 HPTLC chromatogram for various solvent systems of Cannon ball flowers; a- Quercetine

Standard 1 mg / ml, b- Eugenol Standard 1 mg / ml, c- Eugenol Standard 1 mg / ml, d- Canon ball tree as such sample.

RF of different extracts of Cannon ball flower showed in Table 2.

Table 1 RF of different extracts of Cannon ball flower

Sample	Solvent system	No. of peak	Rf values
Quercetine Standard 1 mg / ml	Toluene: Ethyl acetate: Formic acid: Methanol (5.5:3:1:0.5)	1	0.455
Eugenol Standard 1 mg / ml		1	0.650
Eugenol Standard 1 mg / ml		5	0.000, 0.069, 0.143, 0.602, 0.793
Canon ball tree as such sample		1	0.629

3. Anxiolytic Activity Evaluation a. Elevated Plus Maze (EPM)

Table 2 *Courapita guiniasis* extract affects time spent in open arm and entries in elevated plus maze in rats with acute restraint stress-induced anxiety.

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*
<i>Courapita guiniasis</i> 100 mg/kg	106.00±31.92**	16.50±6.43
<i>Courapita guiniasis</i> extract 200 mg/kg	127.67±12.37**	34.67±8.82**

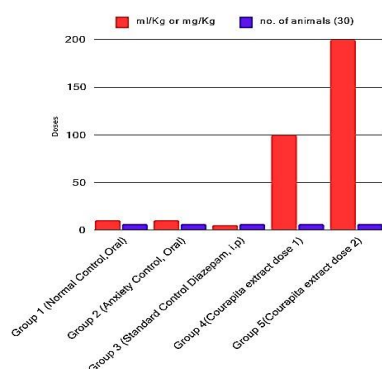


Figure 4 *Courapita guiniasis* extract affects opening arm time and entries in elevated plus maze in rats with acute restraint stress-induced anxiety.

Data were analyzed using one-way ANOVA followed by the Bonferroni post hoc test, with ## $p < 0.001$ in comparison to the normal control group, and ** $p < 0.001$ in comparison to the acute restraint stress group.

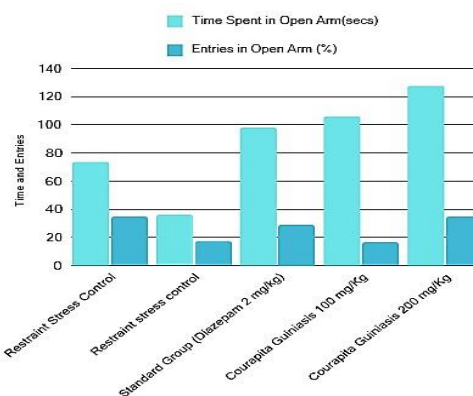


Figure 5 *Courapita guiniasis* extract affects rats' open-arm entries in raised plus mazes during acute restraint stress-induced anxiety.

Data were analyzed using one-way ANOVA followed by the Bonferroni post hoc test, with ## $p < 0.001$ in comparison to the normal control group and ** $p < 0.001$ relative to the acute restraint stress group.

b. Open Field Test (OFT)

Table 3 Effects of *Courapita guiniasis* extract on number of crossings by open filed test in acute restraint stress induced anxiety like behaviour 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*
<i>Courapita guiniasis</i> 100 mg/kg	132.83±28.11

Courapita guiniasis extract 200 mg/kg	115.63±29.43
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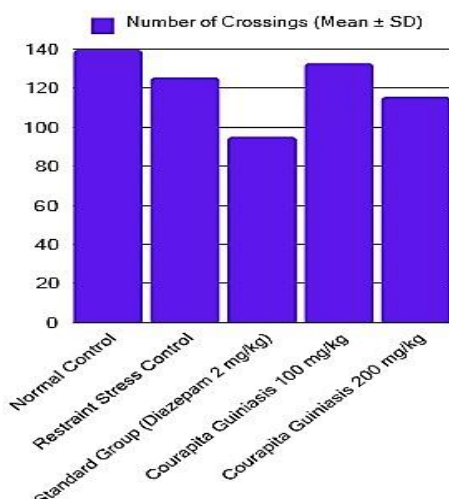


Figure 6 Impact of *Courapita guianensis* extract on the frequency of crossings in the open field test in rats exhibiting anxiety-like Behaviour produced by acute restraint stress.

Data were analyzed using one-way ANOVA, followed by the Bonferroni post hoc test; ## $p < 0.001$ in comparison to the normal control group, and ** $p < 0.001$ relative to the acute restraint stress group.

c. Forced swim test (FST)

Table 4 Effects of *Courapita guiniasis* extract on immobility time by forced swim test in acute restraint stress induced anxiety like behaviour 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**

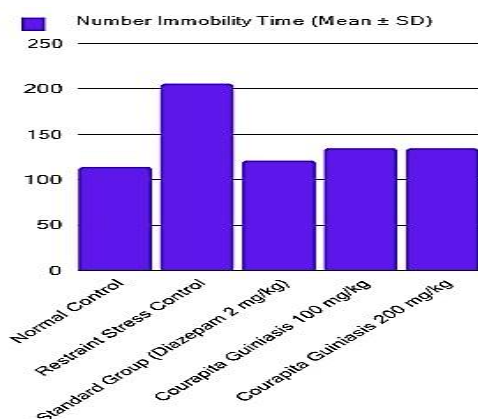


Figure 7 Effects of *Courapita guiniasis* extract on immobility time by forced swim test in acute restraint stress induced anxiety like behaviour in rats.

Data were analyzed using one-way ANOVA followed by the Bonferroni post hoc test, with ## $p < 0.001$ in comparison to the normal control group and ** $p < 0.001$ relative to the acute restraint stress group.

SUMMARY AND CONCLUSION

HPTLC fingerprinting is a reliable and effective diagnostic tool for identifying bioactive

compounds. This study confirmed the presence of the phenolic compound eugenol in the extract of Cannonball Flowers using HPTLC fingerprinting, with eugenol serving as the reference standard. Modern medicine continues to build upon the essential knowledge derived from plants, with nearly all parts of the plant—leaves, flowers, fruits, bark, roots, stems, and seeds—having therapeutic uses. Given the increasing popularity of natural products, extensive research has focused on active plant extracts as potential sources of novel therapeutics. The present study highlights the potential of Cannonball Flower as a reservoir of chemical diversity, which could be valuable for drug screening. *C. guianensis*, in particular, is rich in flavonoids, and further exploration of these flavonoids may uncover interesting antimicrobial properties. The HPTLC method developed here provides a means to analyze various formulations, including eugenol. The systematic application of these scientific methods could help standardize Unani medicine to some extent, enhancing consumer confidence in the use of such products.

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