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Isolation and Characterization of Eugenol from Couroupita Guianensis Flowers and Evaluation of its Anxiolytic Effects in Rats

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Abstract
The aim of the present study was to isolate and purify eugenol from the methanolic extract of the cannaball flower. The methanolic extract of the Cannonball Flower was identified to contain eugenol using HPTLC fingerprinting. To confirm the presence of Eugenol in the Cannonball Flower extract, the easily accessible standard Eugenol was used as a reference marker. This study aims to assess the possible anxiolytic effects of a methanolic extract of Couroupita guianensis flowers (CGFM) in rats. Male Swiss albino rats received 100 and 200 mg/kg of aqueous and methanolic extracts of CG orally for 15 days. The anxiolytic impact was assessed using the Open Field Test (OFT), Forced Swim Test (FST), and Elevated plus Maze (EPM) models. Eugenol was identified in the Cannonball Flower extract at concentrations of 4, 6, and 8 µg/ml, shown as blue bands (T1), (T2), and (T3) in the HPTLC chromatogram at 366 nm. The standard (Eugenol) and the cannonball flower extract were analyzed by HPTLC at concentrations of 4, 6, and 8 µg/ml. Eugenol was identified in the Cannonball Flower extract across all three concentration levels. Compared to the vehicle control, the aqueous and methanolic extracts of CG at a dose of 200 mg/kg demonstrated a substantial anxiolytic effect in mice in the EPM, FST, and OFT models. The anxiolytic properties of CG flower extracts have not been explored.

Keywords: HPTLC, Eugenol, Courapita Guiniasis and Anxiolytic Effect.

1 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).

2 MATERIAL AND METHODS

2.1 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

2.2 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).

2.3 Purification of Plant Extract

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

2.4 HPTLC Analysis

An HPTLC investigation was conducted on the methanolic extract of cannonball flowers. High-performance thin-layer chromatography was performed using silica gel 60F 254 (10 cm × 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 µg/ml before being analyzed by HPTLC at concentrations of 4, 6, and 8 µg/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).

2.5 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).

"Table 1 Grouping 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

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.

3.2 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.

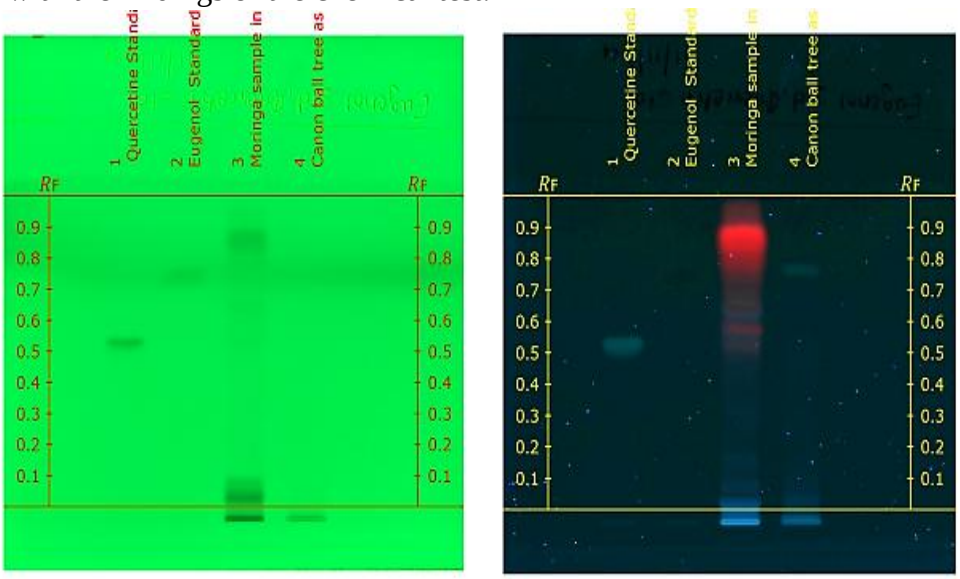
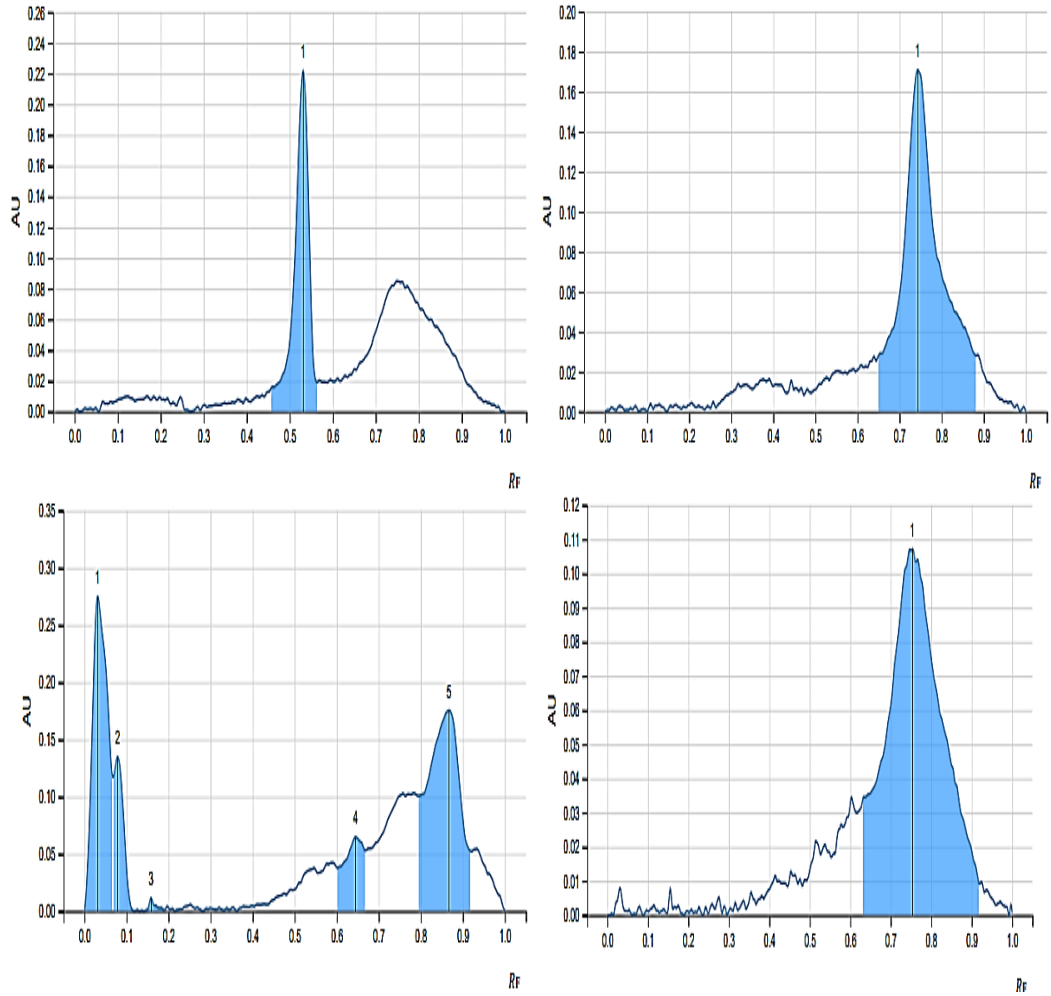


Figure 2 TLC plates scanned at 254 and 366 nm for C. guianensis flower extracts

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 2 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"

3.3 Anxiolytic Activity Evaluation

3.3.1 Elevated Plus Maze (EPM)

"Table 3 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*

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**

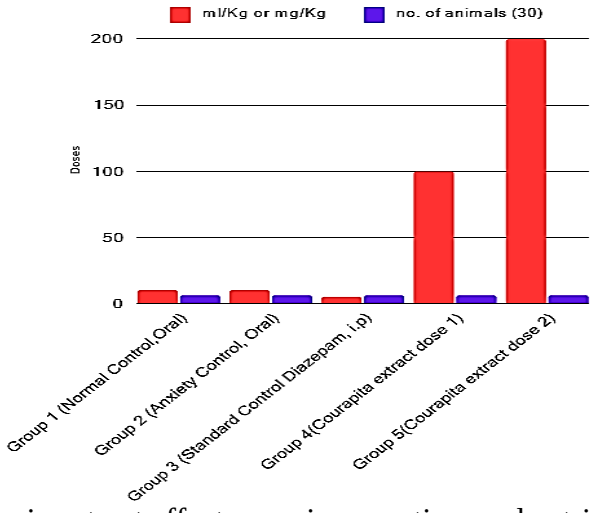


Figure 4 Courapita guiniasis extract affects opening arm time and entries in elevated plus maze in rats with acute restraint stress-induced anxiety.

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.

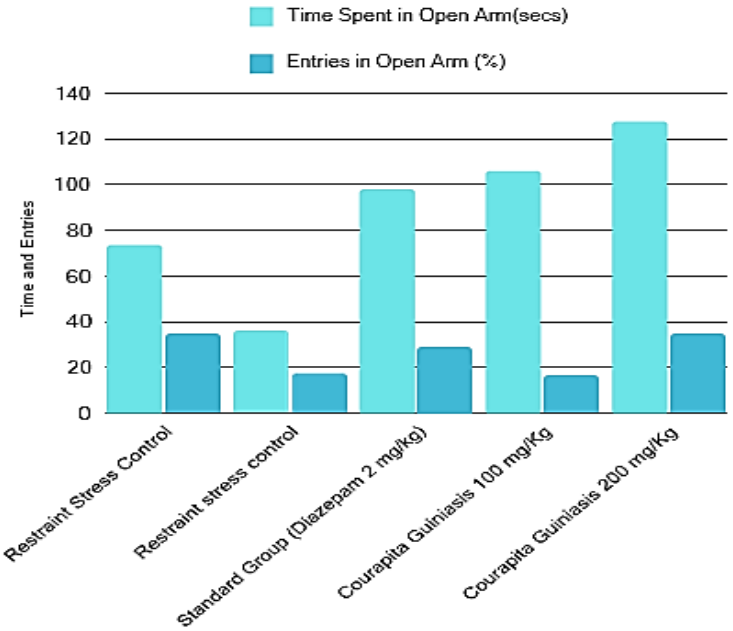


Figure 5 Courapita guiniasis extract affects rats' open-arm entries in raised plus mazes during acute restraint stress-induced anxiety.

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.

3.3.2 Open Field Test (OFT)

"Table 4 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*
Courapita guiniasis 100 mg/kg	132.83±28.11
Courapita guiniasis extract 200 mg/kg	115.63±29.43"

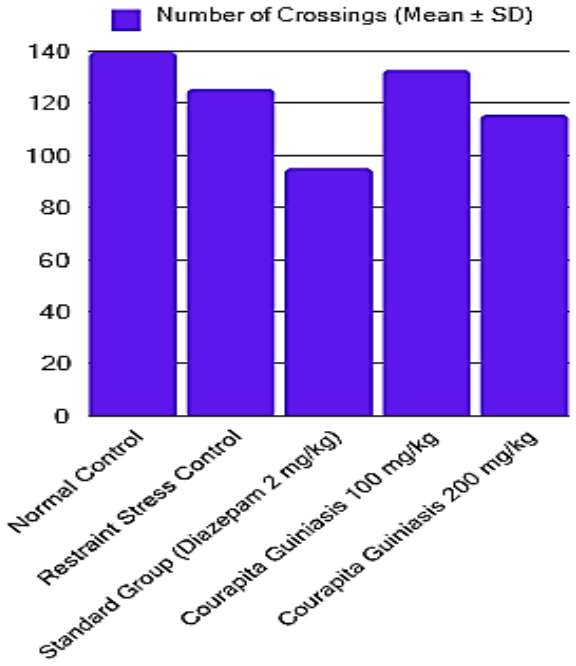


Figure 6 Impact of Courapita guianensis extract on the frequency of crossings in the open field test in rats exhibiting anxiety-like Behaviour produced by acute restraint stress.

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.

3.3.3 Forced swim test (FST)

"Table 5 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**

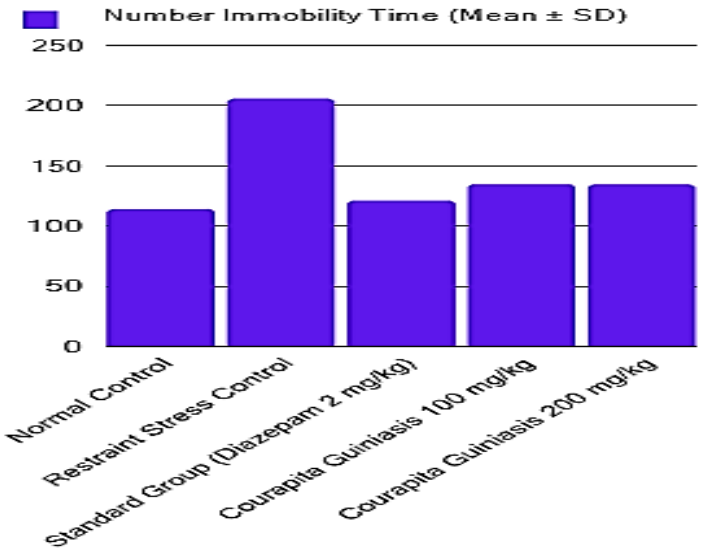


Figure 7 Effects of Courapita guiniasis extract on immobility time by forced swim test in acute restraint stress induced anxiety like behaviour in rats."

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.

3.3.4 Brain model

"Table 6 Effects of Courapita guiniasis extract on GABA levels in acute restraint stress induced anxiety like behaviour 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*

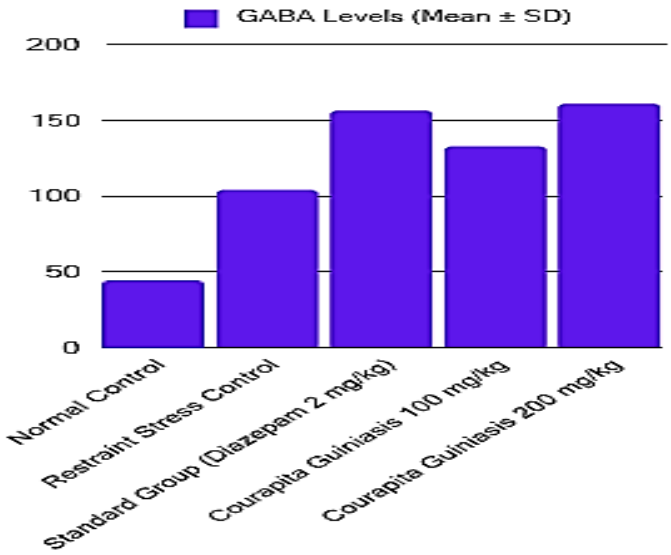


Figure 8 Effects of Courapita guiniasis extract on GABA levels in acute restraint stress induced anxiety like behaviour in rats."

One-way ANOVA and the Bonferroni post hoc test were used to analyses the data; the results showed that the Acute Restrain Stress group was ##p<0.001 compared to the Normal Control group and **p<0.001 compared to the Acute Restrain Stress group.

4 SUMMARY AND CONCLUSION

HPTLC fingerprinting is a reliable diagnostic technique for identifying bioactive chemicals. The research confirmed the existence of the phenolic chemical eugenol in the extract of cannaball flowers (14–16) with HPTLC fingerprinting using eugenol as the reference component. Contemporary medicine is built on essential plant-derived knowledge. Nearly every part of the plant has a therapeutic use, including the leaves, flowers, fruit, bark, roots, stem, and seeds. A considerable body of research has been undertaken on active plant extracts as prospective innovative therapeutics, given the rising popularity of natural goods. Current study indicates that this plant serves as a reservoir of chemical diversity, which will be beneficial for drug screening. The predominant parts of C. guianensis are abundant in flavonoids. Further investigation into the many flavonoids present in C. guianensis may reveal fascinating effects on other microbes (17, 18). This HPTLC approach has been developed to facilitate the measurement of several polyhedral formulations, including eugenol. The systematic use of these scientific methods would facilitate the standardizing of Unani medicine to some degree and would certainly bolster consumer trust in the utilization of these goods (19).

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