A COMPARATIVE PHYTOCHEMICAL AND ANXIOLYTIC SCREENING OF ETHANOLIC EXTRACTS OF LEAVES AND FLOWERS OF *HIBISCUS ROSA SINENSIS* LINN. IN MICE

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Abstract

Anxiety is a medical condition related to our psychological as well as physiological behavior having numerous characters like cognitive, emotional, behavioral, and somatic. The present study was based on a comparative phytochemical and anxiolytic screening of ethanolic extracts of leaves and flowers of *Hibiscus rosa sinensis*. Soxhlet extraction was done for flowers and leaves of *Hibiscus rosa sinensis* using solvent like ethanol at 60°C for 48hr. Phytochemical screening was performed for both the extracts and flavonoids was isolated utilizing TLC, Column chromatography and UV and FTIR spectrophotometry. Elevated plus maze and light/dark arena models were used for the pharmacological screening. In results, the percentage yield of Ethanolic flower and leaves extract of *Hibiscus rosa sinensis* were reported 2.98% & 8.3%, respectively. ethanolic leaves extracts of Hibiscus rosa sinensis showed an excellent anxiolytic activity in comparison to the flower extract of hibiscus rosa sinensis in both the models used- elevated plus maze and light/dark arena model. For flower, in elevated plus maze no. of entries in open arm of HFE (200mg/kg) was 5.26±0.68 and HFE (400mg/kg)7.36±0.72 and time spent in open arm area of HFE (200mg/kg) was 67.30±0.78 and HFE (400mg/kg) was 75.80±0.71 and in light/dark arena model no. of entries in light arena HFE (200mg/kg) was 5.13±0.43 and HFE (400mg/kg) was 7.19±0.58 and time spent in light arena HFE (200mg/kg) was 64.28±0.63 and HLE (400mg/kg) was 77.47±0.81 and for leaf, elevated plus maze in no. of entries in open arm HLE (200mg/kg) at 5.43±0.48 and HLE (400mg/kg) 7.56±0.76 and time spent in open arm area HLE (200mg/kg) at 74.30±0.60 and HLE (400mg/kg) at 76.50±0.81 and in light/dark arena model no. of entries in light arena HLE (200mg/kg) at 5.41±0.63 and HLE (400mg/kg) at 7.46±0.74 and time spent in light arena HLE (200mg/kg) at 65.27±0.18 and HLE (400mg/kg) at 81.30±0.43 were reported. In conclusion, ethanolic leaves extract of *Hibiscus rosa sinensis* has higher content of flavonoids and is an important herbal anxiolytic medication.

Keywords: neuroprotective, neurobehavior, herbal extracts and elevated plus maze, light/dark arena model.

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INTRODUCTION

Anxiety is a complex mental health issue that manifests itself in a variety of ways, including cognitively, emotionally, behaviorally, and somatically [1]. According to available evidence, the a-2 GABA-A subunit plays a key role in reducing anxiety, and current research suggests that the a-3 GABA-A subunit may also be linked to anxiety [2].According to recent data from 2017 about anxiety, 284 million individuals are suffered by this illness globally that includes 63% of females [3].Generalized anxiety disorder which is characterised by persistent worry over a variety of aspects of one's life for at least six months, is uncommonly present without a co-morbid mental disorder. Social phobia is characterised by an irrational and persistent worry in social situations, leading to avoidance of those situations [4]

Anxiety in kids can be more widespread than you think. Babies develop fear of strangers between the ages of 7 and 9 [5]. Benzodiazepines are effective in treating a wide range of anxiety disorders; the GABA pathway's disturbance has been proposed as a possible underlying cause [6].

There are 15 distinct types of Indian agriculture and an estimated 4,700 plant species, of which 15,000 are thought to offer some sort of therapeutic value [7]. Several species of the glabrous shrub Hibiscus rosa-sinensis Linn (Malvaceae) are grown as decorative plants in tropical climates. Yet, the red type is the one most commonly used for medicinal purposes [8].

a. Flower

b. Leaves

Fig 1. Depiction of *Hibiscus rosa sinensis*

The evergreen Hibiscus *rosa sinensis* can either be a shrub or a small tree up to 5 meters in height. There are five or seven lobes on the leaves, each of which is further divided into smaller lobes [9]. The flowers were used to treat epilepsy, diabetes, leprosy, and bronchial catarrh [10].

Taxonomy [11]
Kingdom-Plantae
Sub-kingdom-Tracheobionta
Division-Megnoliophyta
Class-Magnoliopsida
Subclass-Dilleniidae
Order-Malvaceae
Genus-*Hibiscus*
Species-*Hibiscus rosa sinensis*

Chemical constituents

Hibiscus rosasinensis has reported for below mentioned constituents chiefly [12-13]-cyclopropanoids, methyl sterculate, methyl-2-hydroxy sterculate, 2-hydroxysterculate, malvalate, beta-sitosterol. This flower's primary anthocyanin was cyanidin 3-sophoroside and vitamins like Thiamine, niacin, ascorbic acid, riboflavin,and minerals like calcium, phosphorus, iron, and iodoundecane,neopentane,2, 2, 4-trimethyl 3- pentanone,1,2-benzenedicarboxylicacid isodecyl octyl ester,2-cyclopentylethanol,2-propeonic acid,1-4 butanediyl ester,2-propenamide,1-tetrazol-2-ylethanone,4- trifluoroacetoxyoctane, amylnitrite.

Flowers were studied for their potential to treat heart disease in ancient Indian medical texts. Petals were used to treat thinning hair, prevent greying,

and treat and prevent problems of the scalp. As a natural emollient, it was incorporated into hair washes, treatments, and vinegar rinses [13-14]. Therefore, the present study was a comparative phytochemical and anxiolytic screening of ethanolic extracts of leaves and flowers of Hibiscus rosa sinensis in mice.

MATERIALS AND METHODS

Collection and Authentication of the plant

The flowers and leaves of Hibiscus rosa sinensis were collected from Bareilly and authenticated by Department of Plant Science, MJP Rohilkhand University, Bareilly.

Preparation of Hibiscus rosa sinensis extract

Soxhlet extraction: Soxhlet extraction of 20 g plant powder material was done in 150 ml volume using solvent like ethanol.Cycles of extraction were carried out at 60°C for 48hr until all of the dissolved plant material had been completely recovered. For further examination, the extracts were then concentrated in petridishes at room temperature and kept in a refrigerator in airtight bottles. Soxhlet extracts were prepared for both flowers and leaves separately [15].

$$\% \text{ Yield of extract} = \frac{\text{Weight of extract}}{\text{Weight of powder taken}} \times 100$$

Table 1. Color, Consistency and Yield of *Hibiscus rosa sinensis* flowers and leaves Extract

Extracts	Color	Consistency	% Yield
Flower soxhlation extract	Reddish brown	Oily	2.98
Leaf soxhlation extract	Greenish-black	Oily	8.3

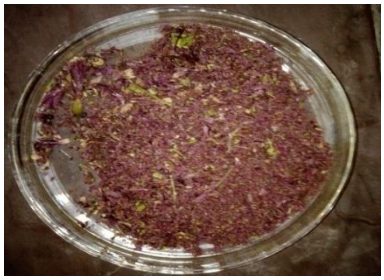

Fig 2. Flower powder of *Hibiscus rosa sinensis*

Fig 3. Leaf powder of *Hibiscus rosa sinensis*

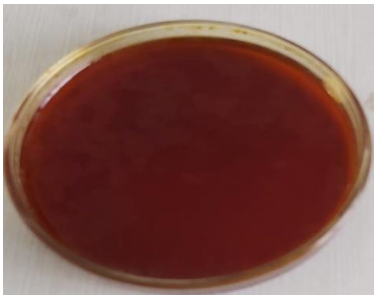

Fig 4. Ethanolic extract of flower *H. rosa sinensis*

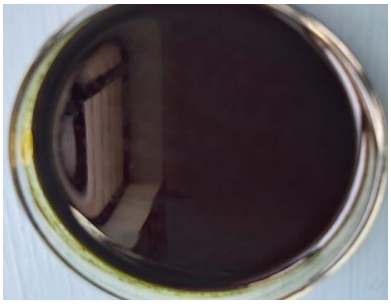

Fig 5. Ethanolic extract of leaf *H. rosa sinensis*

Qualitative screening of phytochemicals in ethanolic flower and leave extracts of *Hibiscus rosa sinensis*

The ethanolic flower and leave extracts of Hibiscus rosa sinensis were subjected to qualitative test using standard procedures to identify various constituents.

Thin layer chromatography

Thin layer chromatography of Hibiscus rosa sinensis flowers and leaves. Thin layer chromatography (TLC) was employed in this study to analyse the nature of compounds present in the crude ethanolic flower and leave extracts of Hibiscus species. Stationary phase silica gel G (scored 10×20 cm) plates, were used.

Table 2. TLC of phytoconstituents

Phytoconstituents	Solvent system
Alkaloids	Toluene: ethyl acetate: diethyl amine (7:2:1)
Steroidal saponin	Ethyl acetate: Ethanol: water: ammonia (6.5:2.5:0.9:0.1)
Triterpenoids	Ethyl acetate: glacial acetic acid: water: formic acid (10:1.1:1.1:2.6)
Tannins	Chloroform: ethyl acetate: ethanol (6:4:4)
Flavones	Ethyl acetate: formic acid: glacial acetic acid: water (10:1.1:1.1:2.6)
Anthocyanin	Ethyl acetate: glacial acetic acid: water: formic acid (10:1.1:1.1:2.6)

A room full of iodine vapour, ninhydrin in acetone at a concentration of 0.2% sprayed, Spraying the plates with a Vanillin-sulphuric acid reagent and observing the results under UV light.
The Rf value of individual spot was calculated by using the following formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Column Chromatography For Ethanolic Extract of Flower and Leaf
Ethanolic extract from the flowers and leaves of *H. rosa sinensis* was adsorbed on silica gel for column chromatography (60–120 mesh). The slurry was loaded on top of the silica gel column that was filled with petroleum ether after being air dried to remove any surface-adsorbed moisture. As the existing/new spot was visible on TLC, the polarity of the column solvent steadily increased with its appearance/disappearance [16].

UV Spectroscopy of Ethanolic flower and leaf extracts of Hibiscus rosa sinensis
Hibiscus rosa sinensis flower and leaf extracts were dissolved in ethanol in microgram amounts. Shimadzu 1601 UV-spectrophotometer was used to scan their UV-visible spectra between 200 and 800 nm. Each time, the base line was set against the solvent used to make the specific extract solution. Peaks of maximum absorption were noticed in the scanned spectra, which were recorded [17].

FT-IR fingerprinting in Ethanolic flower and leaf extracts of Hibiscus rosa sinensis:
Each Hibiscus rosa sinensis (10 mg) flower and leaf extract was combined with 100 mg of dry potassium bromide (KBr) and compressed to create a salt disc. The disc was then spectrophotometrically read using a Shimadzu FTIR-8101 spectrophotometer in the 400–4000 cm-1 range (Vmax in cm-1). Each extract's frequencies of the various constituents were examined [18].

Preparation of animals
For the investigation, male albino mice weighing 60-80g were obtained from the Animal house, Department of Pharmacy MJPRU Bareilly. The animals were always kept in colony cages with free access to food and water, under typical climatic conditions of 24± 20ºC temperature, a 12:12 h light/dark cycle, and 30- 70% relative humidity. The animals were left without food overnight and throughout the experiment. The protocol for the investigation on anxiolytic action was approved by the institutional animal ethical committee [19].

Group design
Swiss mice weighing b/w 60-80g were divided into 4 groups each containing 6 animals-

Table 3. Group for Hibiscus Rosa sinensis flower

S. N.	Treatment	Dose
Group 1	Control	0.5 ml 1% CMC solution (i.p.)
Group 2	Standard (Diazepam)	1.5mg/kg, (i.p.)
Group 3	HRE (flower)	200mg/kg, (orally)
Group 4	HRE (flower)	400mg/kg, (orally)

Table 4. Group for Hibiscus Rosa sinensis leaves

S. N.	Treatment	Dose
Group 1	Control	0.5 ml 1% CMC solution (i.p.)
Group 2	Standard (Diazepam)	1.5mg/kg, (i.p.)
Group 3	HLE (Leaf)	200mg/kg, (orally)
Group 4	HLE (Leaf)	400mg/kg, (orally)

Protocols
i. Elevated plus maze apparatus
Using transfer latency, the Elevated plus maze (EPM) test has been proposed as a straightforward way for assessing learning and memory in mice. The exteroceptive behavioural model (EPM) used here assumes that stimuli are external to the organism. To create the plus sign shape, we used an elevated plus maze with two open arms (16cm x 5 cm) and two enclosed arms (16cm x 5cm x 12 cm) connected to each other. The maze's arms spread forth from a 5cm x 5cm platform at a height of 25cm off the ground. On day seven of drug administration, we positioned each mouse on its own individual open arm, with its back to the centre platform. The time it took for the mouse to hop into one of the enclosed arms was recorded as the transfer latency. Each animal's TL was recorded on

day one. After 2 more minutes of maze exploration, the mouse was put back in its home cage. 24 hours after the first day of trial (the eighth day of medication treatment), participants were tested on their ability to recall the steps of this newly learnt activity [20].

ii. Light/dark arena model

Basically, it's a wooden box with a lid that's used as an instrument. A distance of 25 centimeters above the top of the open box was occupied by two chambers, one painted black (25 cm long x 35 cm broad x 35 cm deep) and made dark by covering its top with black plywood, and the other painted white (25 cm long x 35 cm wide x 35 cm deep) and extremely bright with a 40-W white light source. A small open doorway (7.5 cm length 5 cm wide) on the floor level in the middle of the divider united the two rooms.The procedure involves letting the animal roam freely within the model while keeping track of how many times it enters and exits the light field and how long it stays there[21].

Statistical analysis

Statistics were reported using a mean standard error of the mean format. Mean comparisons were performed using one-way analysis of variance and Tukey's test. Statistics were significant if $P \leq 0.001$.

RESULTS& DISCUSSION

Physical properties of flower and leave extracts of *Hibiscus rosa sinensis*

The dried flower and leaf powder of *Hibiscus rosa sinensis* were reported the percentage yield of 59.45% & 63.62% respectively.

Table 5. Physical properties of the flower and leaf extract of *Hibiscus rosa sinensis*

Physical characteristics	Ethanolic flower extract	Ethanolic leaf extract
Color	Reddish brown	Greenish black
Odor	Characteristic	Characteristic
Consistency	Oily	Oily
% Yield	59.45%	63.62

Both the leaves and flowers were evaluated for their phytochemical's content. They showed a rich source of alkaloids, terpenoids, coumarins etc. when tested using different parameters.

Table 6. Phytochemical analysis in Ethanolic flower and leaf extracts of *Hibiscus rosa sinensis*

Phytochemicals	H.R.S flower extract	H.R.S leaf extract
Alkaloids	+	+
Carbohydrates	-	-
Triterpenoids	+	-
Coumarins	+	-
Steroids	-	+
Tannins	+	-
Saponins	+	-
Flavones	+	+
Chalcones	-	+
Amino acids	-	-
Glycosides	-	-
Proteins	-	-
Phenols	-	-

(+) = Indicates presence (-) = Indicates absence

Table 7 shows the proximate analysis of the extracts as below-

Table 7. Proximate analysis of *Hibiscus rosa sinensis* flowers and leaves-

Parameters	Value% (w/w) (flower)	Value% (w/w)(leaf)
Ash values		
Total Ash value of crude drug	5.5	14
Acid insoluble ash	2	5.5
Water soluble ash	1.5	0.5
Sulphated ash	13	9
Loss on drying	78	86
Extractive values		
Water soluble extractives	33.6	36
Alcohol soluble extractives	18.4	9.6

It showed that total ash content was higher in leaves when compared with flowers. But water-soluble extractives were highest as well as alcohol soluble extractives in flowers when compared with leaves.

TLC of *Hibiscus rosa sinensis* extracts

The following tables detail the outcomes of creating and visualizing agents used to create the TLC chromatographic profile of ethanolic flower and leaf extracts of Hibiscus rosa sinensis.

TLC chromatogram in ethanolic flower and leaf extracts of Hibiscus rosa sinensis-

Table 8.TLC observations of ethanolic flower extract of *Hibiscus rosa sinensis*

Phytoconstituent	Mobile phase	Color	Rf Value
Flavonoid	Ethyl acetate: Formic acid: Glacial acetic acid: H_2O (100:11:11:26)	Yellowish green	0.72

Table 9. TLC observations of ethanolic leaves extract of Hibiscus rosa sinensis

Phytoconstituent	Mobile phase	Color	Rf Value
Flavonoid	Ethyl acetate: Formic acid: Glacial acetic acid: H_2O (100:11:11:26)	Green	0.86

UV analysis was being represented as below in Fig 6. & 7 of ethanolic flower extract and ethanolic leaves extract, respectively.

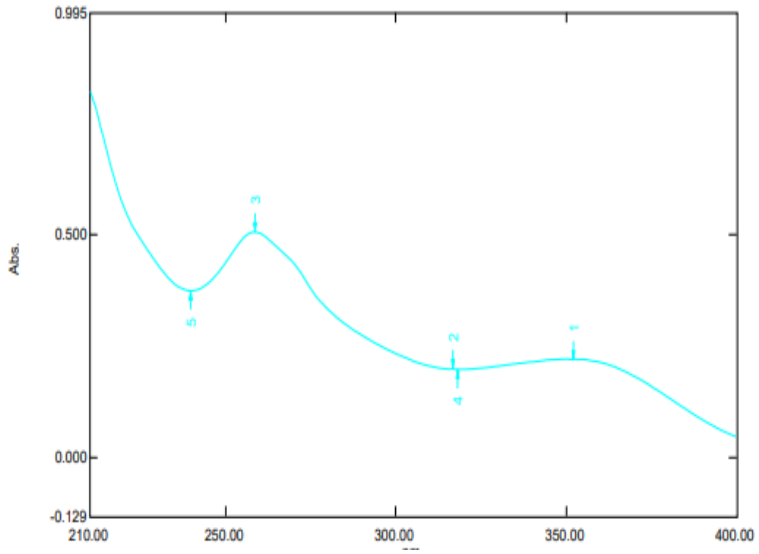

Fig 6. UV analysis of Ethanolic extract of flower of Hibiscus rosa sinensis

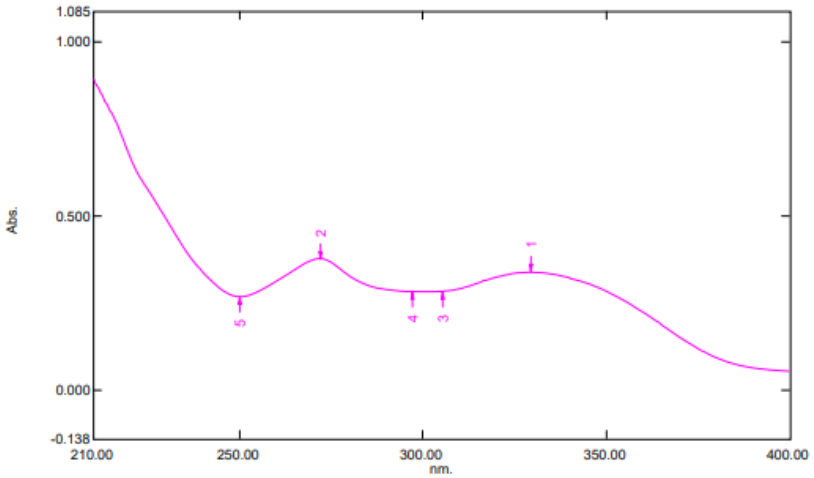

Fig 7. UV analysis of Ethanolic extract of leaves of Hibiscus rosa sinensis

Fig 8. and 9 demonstrates the FTIR data of extracts of flower and leaves as mentioned below-

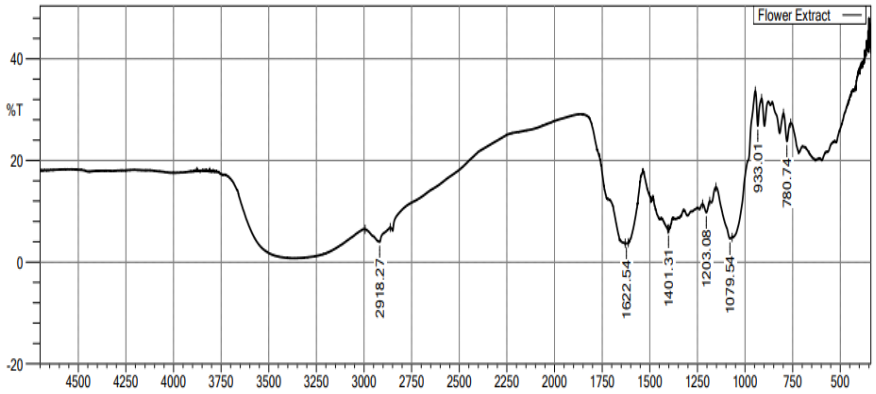

Fig 8. FTIR of Ethanolic extract of flower of Hibiscus rosa sinensis

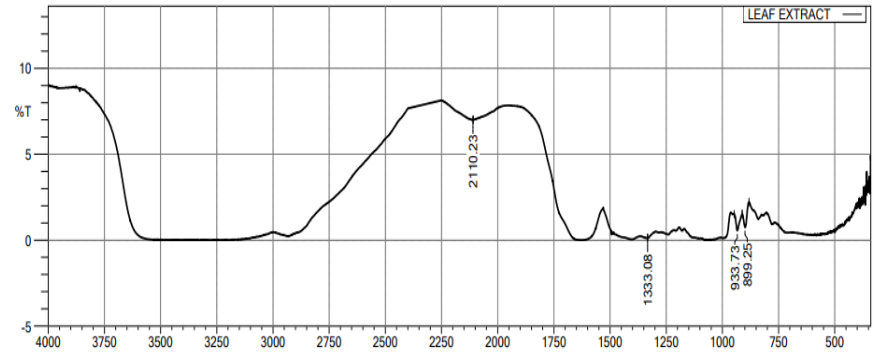

Fig 9. FTIR of Ethanolic extract of leaves of Hibiscus rosa sinensis

SCREENING OFANXIOLYTIC ACTIVITY

i. Elevated Plus Maze Test

Ethanolic Flower extracts of Hibiscus rosa sinensis (HFE)

The no. of entries was observed as 5.26±0.68 and 7.36±0.72 in HFE (200mg/kg) and HFE (400mg/kg) treated groups, respectively. Thus, it showed that no. of entries was better modulated in test groups when compared with the control group.

However, the standard drug- Diazepam treated animals exhibited an excellent no. of entries and action of higher dose was found near the Diazepam. Thus, it proved for its anxiolytic efficacy.

The following table represents the no. of entries of control, standard and HFE-

Table 1. Effect of Control, standard and HFE on No. of entries in open arm

Treatment	**No. of entries in open arm (Mean± SEM)**
Control (0.5ml, 1%)	4.23±0.49
Diazepam (1.5mg/kg)	8.69±0.72
HFE (200mg/kg)	5.26±0.68
HFE (400mg/kg)	7.36±0.72

Significance level was demonstrated at $p<0.05$

Values shown in Mean± SEM

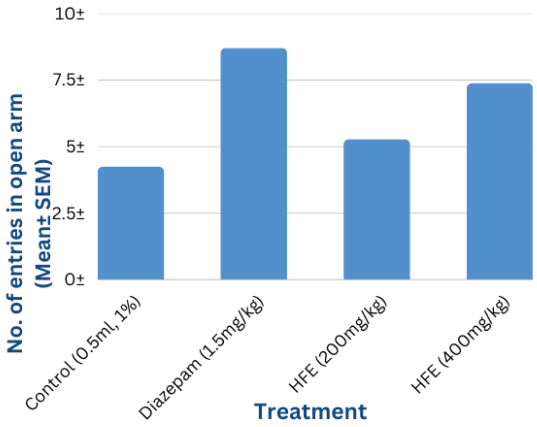

Fig 1. Graphical data of effect of control, standard and HFE on No. of entries in open arm

Table 2. shows the effect of Control, standard and HFE on time spent in open arm using elevated plus maze apparatus.

Table 2. Effect of Control, standard and HFE on time spent in open arm

Treatment	**Time spent in open arm (Mean± SEM)**
CMC 0.5ml (1%)	25.23±0.40
Diazepam (1.5mg/kg)	78.6±0.72
HFE (200mg/kg)	67.30±0.78
HFE (400mg/kg)	75.80±0.71

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

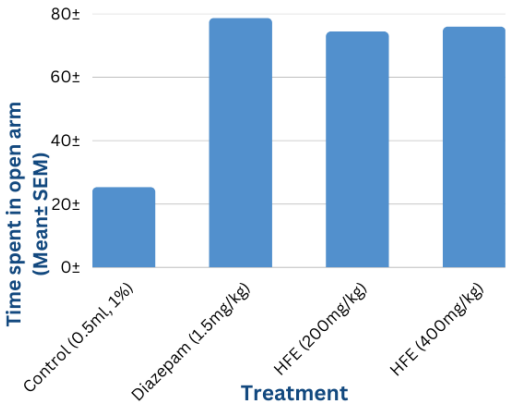

Fig 2. Graphical data of effect of Control, standard and HFE on time spent in open arm

Time spent was observed as 67.30±0.78 and 75.80±0.71 in HFE (200mg/kg) and HFE (400mg/kg) treated groups, respectively. Thus, it showed that time spent was better modulated in test groups when compared with the control group.
However, the standard drug- Diazepam treated animals exhibited an excellent time spent and action of higher dose was found near the Diazepam. Thus, it proved for its anxiolytic efficacy.

Ethanolic Leaves extracts of *Hibiscus rosa sinensis* (HLE)

In order to determine the anxiolytic potential of ethanolic leaves extract of *Hibiscus rosa sinensis* (HFE), mice were divided into 4 different groups. Group 1 was served as control that was fed with 1% CMC. Group 2 was given Diazepam (1.5mg/kg) and served as standard. Whereas, group 3 was administered ethanolic leaves extract of *Hibiscus rosa sinensis* (HLE) at the dose of 200mg/kg and group 4 administered ethanolic leaves extract of

Hibiscus rosa sinensis (HLE) at the dose of 400mg/kg. All the treatments were proceeded once a day for 21 days.

The following table represents the no. of entries of control, standard and HLE-

Table 3. Effect of Control, standard and HLE on No. of entries in open arm

Treatment	No. of entries in open arm (Mean± SEM)
CMC 0.5ml (1%)	4.39±0.47
Diazepam (1.5mg/kg)	9.39±0.72
HLE (200mg/kg)	5.43±0.48
HLE (400mg/kg)	7.56±0.76

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

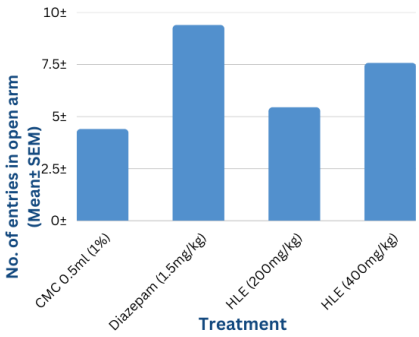

Fig 3. Graphical data of effect of Control, standard and HLE on No. of entries in open arm

In leaves extract, the no. of entries was observed as 5.43±0.48 and 7.56±0.76 in HLE (200mg/kg) and HLE (400mg/kg) treated groups, respectively. Thus, it showed that no. of entries was better modulated in test groups when compared with the control group. While the control group showed minimum no. of entries.

However, the standard drug- Diazepam treated animals exhibited an excellent no. of entries and action of higher dose of leaves of *Hibiscus rosa sinensis* was found near the Diazepam. Thus, it proved for its anxiolytic action.

Table shows the effect of Control, standard and HFE on time spent in open armusing elevated plus maze apparatus. Time spent was observed as 74.30±0.60 and 76.50±0.81 in the group treated with HLE of *Hibiscus rosa sinensis* at the dose of 200mg/kg and 400mg/kg, respectively.

Therefore, it indicated for anxiolytic role in mice.

Table 4. Effect of Control, standard and HLE on time spent in open arm

Treatment	Time spent in open arm (Mean± SEM)
CMC 0.5ml (1%)	36.27±0.40
Diazepam (1.5mg/kg)	84.6±0.72
HLE (200mg/kg)	74.30±0.60
HLE (400mg/kg)	76.50±0.81

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

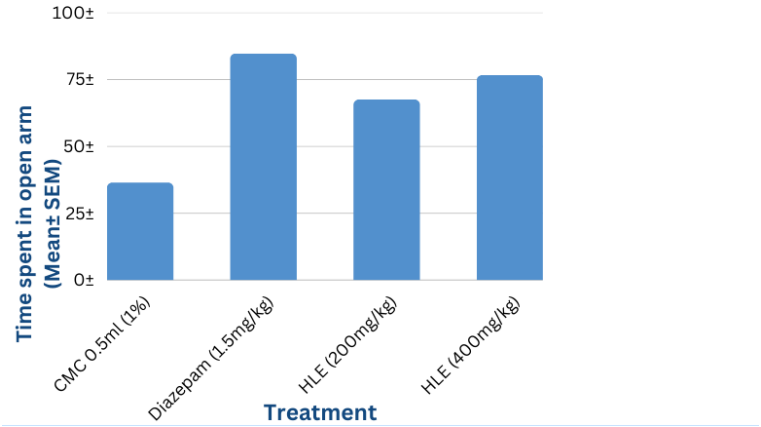

Fig 4. Graphical data of effect of Control, standard and HLE on time spent in open arm

ii. Light/Dark Arena Test

Ethanolic Flower extracts of *Hibiscus rosa sinensis* (HFE)

In light/dark arena model, the no. of entries was observed as 5.41±0.63 and 7.19±0.58 in HFE (200mg/kg) and HFE (400mg/kg) treated groups, respectively. Thus, it showed that no. of entries was better modulated in test groups when compared with the control group. However, the standard drug- Diazepam treated animals exhibited an excellent no. of entries and action of higher dose was found near the Diazepam. Thus, it proved for its anxiolytic efficacy. The following table represents the no. of entries of control, standard and HFE-

Table 5. Effect of Control, standard and HFE on No. of entries in light arena

Treatment	No. of entries in light arena (Mean± SEM)
CMC 0.5ml (1%)	4.27±0.69
Diazepam (1.5mg/kg)	8.29±0.42
HFE (200mg/kg)	5.13±0.43
HFE (400mg/kg)	7.19±0.58

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

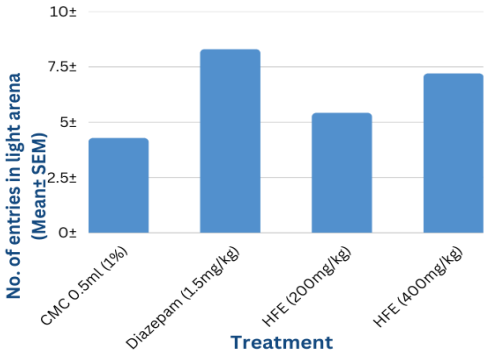

Fig 5. Graphical data of effect of Control, standard and HFE on No. of entries in light arena

Time spent was recorded as 64.28±0.63 and 77.47±0.81 in the Test 1 (HFE, 200mg/kg) and Test 2 (HFE, 400mg/kg), respectively. Thus, it showed that time spent was better modulated in test groups when compared with the control group.

However, the standard drug- Diazepam treated animals exhibited an excellent time spent and action of higher dose was found near the Diazepam. Thus, it proved for its anxiolytic efficacy. Table shows the effect of Control, standard and HFE on time spent in open arm using elevated plus maze apparatus.

Table 6. Effect of Control, standard and HFE on time spent in Light arena

Treatment	Time spent in Light arena (Mean± SEM)
CMC 0.5ml (1% CMC)	27.53±0.48
Diazepam (1.5mg/kg)	81.91±0.42
HFE (200mg/kg)	64.28±0.63
HFE (400mg/kg)	77.47±0.81

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

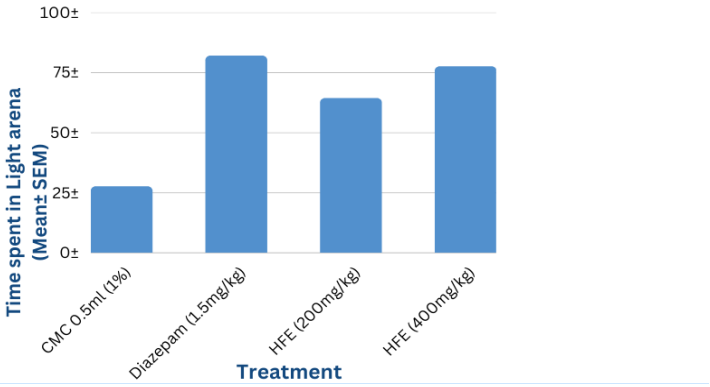

Fig 6. Graphical data of effect of Control, standard and HFE on time spent in Light arena

Ethanolic Leaves extracts of *Hibiscus rosa sinensis* (HLE)

In context to determine the anxiolytic potential of ethanolic leaves extract of *Hibiscus rosa sinensis* (HFE), mice were divided into 4 different groups. Group 1 was served as control that was fed with 1% CMC. Group 2 was given Diazepam (1.5mg/kg) and served as standard. Whereas, group 3 was administered ethanolic leaves extract of *Hibiscus rosa sinensis* (HLE) at the dose of 200mg/kg and group 4 administered ethanolic leaves extract of *Hibiscus rosa sinensis* (HLE) at the dose of 400mg/kg. All the treatments were proceeded once a day for 21 days. However, the control group showed 4.79±0.43 (no. of entries) which was lowest. The following table represents the no. of entries of control, standard and HLE-

Table 7. Effect of Control, standard and HLE on No. of entries in light arena

Treatment	No. of entries in open arm (Mean± SEM)
CMC 0.5ml (1%)	4.79±0.43
Diazepam (1.5mg/kg)	8.82±0.71
HLE (200mg/kg)	5.41±0.63
HLE (400mg/kg)	7.46±0.74

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

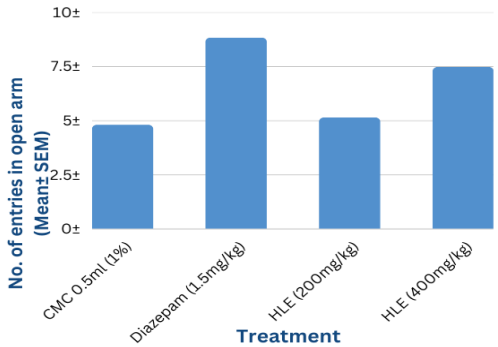

Fig 7. Graphical data of effect of Control, standard and HLE on No. of entries in light arena

Leaves extract demonstrated a better anxiolytic activity when compared with flower extract. No. of entries was observed as 5.41±0.63 and 7.46±0.74 in Test 1 (HLE, 200mg/kg) and Test 2 (HLE, 400mg/kg) treated groups, respectively.

Table 8. Effect of Control, standard and HLE on time spent in open arm

Treatment	Time spent in open arm (Mean± SEM)
CMC 0.5ml (1%)	31.29±0.42
Diazepam (1.5mg/kg)	82.46±0.63
HLE (200mg/kg)	65.27±0.18
HLE (400mg/kg)	81.30±0.43

Significance level was demonstrated at $p<0.05$
Values shown in Mean±SEM

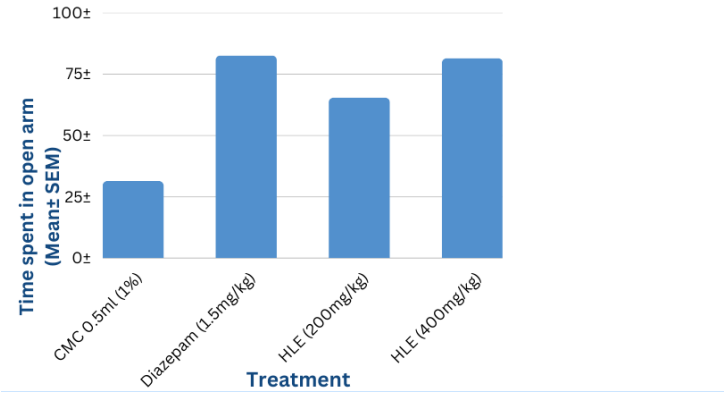

Fig 8. Graphical data of effect of Control, standard and HLE on time spent in open arm

Time spent was found as dose-dependent; increasing with the dose of extract. The time spent was observed highest as 81.30±0.43 in Test 2 (HLE, 400mg/kg) treated mice when compared with control group.

Several studies have confirmed herbal supplements are safe, but no mention has been made of any possible drug interactions. Herbs like black cohosh, chasteberry chamomile and rhodiola are known to modulate the actions of cytochrome P450 enzymes and may increase the toxicity or decrease therapeutic effects of substrate drugs [22]. This includes many drugs. The clinical significance of these interactions is unknown, but the possibility exists, so it is important to give it thought. Herbs with anticoagulant/antiplatelet properties, such as chamomile and lavender may increase the risk of bleeding when combined with drugs with similar actions. Among these are the anticoagulants warfarin and heparin, which are frequently administered to bedridden cancer patients to treat or prevent deep vein thrombosis [23]. The ethanolic leaves and flower extracts of Hibiscus rosa sinensis showed an excellent anxiolytic activity in both the models used- elevated plus maze and light/dark arena model. It is fairly normal for people to experience depression, and for many, it is a persistent problem that interferes with work and family obligations. The motivation, energy, and pleasure needed to support and maintain social, marital, and parental interactions are entirely disrupted. It is a disease with many faces that can manifest at any age, be chronic or waxing and waning, and frequently coexists with a wide range of other problems, including anxiety disorders, substance abuse, and behavioral disorders. There is a lot of information on the prevalence and symptoms of depression in the general population, but less information is available on depression among parents and other carers. It is frequently held responsible for or a contributing factor in medical disorders.

For flower, in elevated plus maze no. of entries in open arm of HFE (200mg/kg) was 5.26±0.68 and HFE (400mg/kg)7.36±0.72 and time spent in open arm area of HFE (200mg/kg) was 67.30±0.78 and HFE (400mg/kg) was 75.80±0.71 and in light/dark arena model no. of entries in light arena HFE (200mg/kg) was 5.13±0.43 and HFE (400mg/kg) was 7.19±0.58 and time spent in light arena HFE (200mg/kg) was 64.28±0.63 and HLE (400mg/kg) was 77.47±0.81 and for leaf, elevated plus maze in

no. of entries in open arm HLE (200mg/kg) at 5.43±0.48 and HLE (400mg/kg) 7.56±0.76 and time spent in open arm area HLE (200mg/kg) at 74.30±0.60 and HLE (400mg/kg) at 76.50±0.81 and in light/dark arena model no. of entries in light arena HLE (200mg/kg) at 5.41±0.63 and HLE (400mg/kg) at 7.46±0.74 and time spent in light arena HLE (200mg/kg) at 65.27±0.18 and HLE (400mg/kg) at 81.30±0.43 were reported.

In conclusion, ethanolic leaves extract of *Hibiscus rosa sinensis* show better anxiolytic activity as compare to ethanolic flower extract due to higher concentration of flavonoids in leaf and also is an important herbal anxiolytic medication. After successfully investigating the plant leaf and flower by phytochemical screening like TLC, Column, UV and FTIR of both flowers and leaves, it can be utilized to treat depression, mental agitation, and other neurological illnesses in future on the basis of presence of flavonoids in higher concentration responsible for antianxiety activity.

It suggests to isolate the concerning moiety/active constituent for antianxiety activity. It would be a great change towards allopathic medicines to counter the mental disorders i.e., anxiety, depression etc.

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CONFLICT OF INTEREST
'None conflict of interest' was declared by the authors.

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