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Research Article

Evaluation of Anti-depressant activity of *Hibiscus rosa sinensis* leaves in mice

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Abstract

Objective: The present is to evaluate antidepressant activity of ethanolic extract of *Hibiscus rosa sinensis* leaves using experimental animals.

Method: The current study employed male Swiss albino mice weighing between 20 and 35 g and aged between 6 and 8 weeks. Hibiscus rosa sinensis extract were administered p.o to mice daily in two different doses (200 & 400 mg/kg/p.o) for 14 days regularly. After administration of the extract, the animal will be observed continuously for the first 2 hours and at 24 hrs to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and also will be monitored up to 14 days for the toxic symptoms and mortality. On 15th days, the animal subjected to Tail Suspension test (TST) and forced swim test (FST) and plasma corticosterone level.

Result: The mice of *Hibiscus rosa sinensis* extract (200 & 400 mg/kg/p.o) treated group showed no significantly (p<0.05) difference in locomotors activity as compared to control group. Treatment with FXT (10 mg/kg p.o.)also showed no significant change as compared to control group. The above observation suggests that *Hibiscus rosa sinensis* leaves has antidepressant activity.

Conclusion: The Ethanolic extract of Hibiscus rosa sinensis 400 mg/kg showed the most remarkable activity. This plant can be further subjected to isolation of the therapeutic Antidepressant compound and carry out further pharmacological evaluation.

Keywords: Depression, herbal medications. *Hibiscus rosa sinensis*, antidepressant plants

INTRODUCTION

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest, also referred to as Major Depressive Disorder or Clinical Depression.[1] The medicines achieve their by negatively affecting the central nervous system (CNS) and brain concentrations of neurotransmitters including NE, serotonin, and dopamine. Based on the mode of actions, a group of antidepressants contain 17 substances which can be further divided into subgroups[2].

According to some, the primary symptom of depression is anhedonia, which is defined as a lack of interest in or enjoyment from activities that are often joyful to people.[3], [4] The first-generation antidepressants, the tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs), increase the concentrations of 5-HT and/or NE and are useful in reducing depression's symptoms. TCAs acts on many other transmitter systems in the CNS and periphery, eg, the histaminergic or acetyl cholinergic systems.[5] China rose or “Queen of tropics” is often a popular name for the gorgeous flowering plant Hibiscus rosa-sinensis.[6] *Hibiscus rosa sinensis* commonly known as Gurhal is large shrub belonging to family malvaceae.[7]

Chemical Constituents: The edible portion of the flower was reported to have the following nutrients like nitrogen, fats, crude fibers, calcium, phosphorus, iron, thiamine, ascorbic acid and niacin. Petals of Hibiscus rosa-sinensis were reported to contain quercetin-3-di-O-β-D-glucoside; quercetin-3-7-di-O-β-D–glucoside, quercetin and kaempferol.[8-10]

Leaves and stems contain β-sitosterol, stigma sterol, taraxeryl acetate and three cyclopropane compounds and their derivatives. Fatty acids, fatty alcohols and hydrocarbons were identified from Hibiscus rosa Sinensis leaves.[8,9,11,12]

It is having Pharmacological Activites like Antimicrobial Activity, Anti-inflammatory Antipyretic Activity Neuroprotective Activities and anxiolytic effect.[13,14]

MATERIAL AND METHOD

Methods of collection of data

Study through research articles, research data based like Medline.

The data related to physicochemical details of the drug will be collected from drug information centre, various

standard books, journals and other sources like literature data bases such as science direct etc.

The data was collected based on laboratory animal experimentation.

Experimental Animals

Albino mice (25-30 gm) of either sex were used in the present study and were housed under standard conditions of light and dark cycle in the central animal house facility of St. Soldier Institute of Pharmacy, Jalandhar, Punjab in different polypropylene cages with husk bedding and were maintained at standard laboratory pellet chow diet and water *ad libitum*. Before the experimental investigation, the animals were adjusted to the lab environment. All experiments were performed between 08:00 and 16:00 hr in semi sound proof laboratory conditions. Approved by the Ministry of Environment and Forests, Government of India's Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (Reg. No. 2011/PO/Re/S/18/CPCSEA, registration date: 1/5/2018), the experimental protocol was conducted in compliance with the guidelines for the use and care of experimental animals. Adequate measures were taken to minimize pain or discomfort with animal's experimental procedure. Research protocol is duly approved by IAEC/CPCSEA (IAEC/SSIP/2022/PR-022).

Drugs and Reagents/ Chemical:

All the chemicals and biochemical reagents used in this study were of analytical grade and were freshly prepared before use. All chemicals of analytical grade were procured from Sigma chemical, USA and S. D. Fine Chem. Ltd., India.

Procurement of the extract:

Hibiscus rosa sinensis leaves ethanolic extract was collected from the dealer Shreedha Phyto extracts Jaipur – 302019 in the month of December 2020. The same group also provided a certification of the Plant's identify and quality.

Experimental animals:

Twenty Four adult either sex mice weighing between 25-30g were obtained from the animal house of Pharmacology Department. ST. Soldier institute of pharmacy, Jalandhar- Amritsar Bypass NH-I , Behind NIT Jalandhar , Punjab India – 144011 .

Animals:

Healthy adult Swiss albino mice, weighing between 25 and 30 g, will be housed in standard laboratory conditions at 25 ± 2°C with a 12-hour light/dark cycle for the purpose of the experiment. There will be food and water for free.

Acute oral toxicity study [1]:

Acute toxicity study for the ethanolic extract of *Hibiscus rosa sinensis* leaves done according to the OECD guidelines No: 423 and low, medium and high dose will be selected for treatment.

Method:

The overnight fasted mice will be divided into 04 groups, each group consisting of 06 animals. The *Hibiscus rosa sinensis* leaves will be given in various doses (100, 2500, 500 and 1000 mg/kg) by oral route with a gavage. After administration of the extract, the animal will be observed continuously for the first 2 hours and at 24 hrs to detect changes in behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and also will be monitored up to 14 days for the toxic symptoms and mortality.

TREATMENT:

GROUPS (where N=4)	Treatment
Group I	Naive animal, received standard pellet diet and tap water ad libitum daily.
Group II	Standard group received 10mg/kg Fluoxetine orally daily.
Group III	Test group-I received 200mg/kg Ethanolic extract of *Hibiscus rosa sinensis* leaves orally daily.
Group IV	Test group –II received 400mg/kg Ethanolic extract of *Hibiscus rosa sinensis* leaves orally daily.

Ethanolic extract of *Hibiscus rosa sinensis* leaves *(EEHRSL)*

Total No. of animals required:-

No. of the animal in each group (n) = 06

No. of groups (N) = 04

Total no. of animals required = 24

Note: All the parameters will perform with suitable time interval to prevent unwanted stress in animals.

Parameters for depression in mice :-

The behavioral effects of an acute or sub acute (14 day course) will be orally administered. *Hibiscus rosa sinensis* leaves (200, 400 mg/kg) ethanolic extract will be evaluated in male and female Swiss mice in Locomotion activity , forced swim test (FST) and Tail Suspension test (TST). The animals were housed in groups of ten in plastic cages with unrestricted access to food and water and maintained at a constant temperature of around 21 +/- 1 ~C. They were kept on an artificial 12 h/12 h day/night cycle.

1. Locomotors activity [15]

Using an actophotometer, which runs on photoelectric cells coupled in circuit with a counter, it is simple to quantify the locomotor activity. A count is kept when the animal cuts off the light beam landing on the photocell. An actophotometer could have either circular or square across in which the animal moves. Both mice and rats may be used for testing in this equipment. **(**Springer, H. Gerhard Vogel)

Figure 1: Actophotometer

2. Forced swimming test[15] :

A 2L glass cylinder was filled with water at room temperature (approximately 25 ± 2o C). The cylinder has a diameter of 10 cm and stands 19 cm tall. It is filled with tap water to a level of 13 cm, approximately 1600 mL, and allowed overnight to reach room temperature. Mice should not be able to touch the bottom of the cylinder with their tails, as this may alter their behavior. Mice are picked up from their home cages in a plastic container with holes in the bottom to let out water, and are individually dropped (placed in, head downward, trying to ensure that the mouse's head does not go underwater) into the glass cylinder and observed for immobility for one 6- min trial.

3. Tail suspension test [16] :-

The method is based on the discovery that agitation and restlessness alternate in a mouse dangling by its tail. For these experiments, the recording device was as follows: metallic gallows were connected to a nylon catheter (d = 1.5 ram, length = 350 ram) with a hook attached to its extremity. There were 350 millimeters separating the device's floor and the hook. An adhesive tape 20 mm from the mouse's tail tip was used to hang it on the hook.

The mouse was separated visually and acoustically at a distance of 150 mm from the closest item. The articulated stylus of the gallows was connected to a Marey capsula that transmitted any pressure difference to another capsula by a pneumatic connection. The receiver capsula was connected to a drawing stylus, marking on a cylinder covered with black smoke. With the aid of an electric motor, the cylinder turned at a speed of 2 cm/min. This apparatus offered an analog documentation of the mouse's motions.

STATISTICAL ANALYSIS

All the results were expressed as Mean ± SEM. The data of all the groups were analyzed by One way ANOVA followed by Turkey's test using software Graph pad prism In Stat (Graph Pad Software Inc., USA). A value of $p<0.05$ was considered to be significant.

RESULTS

H. rosa sinensis L. leaves

Extract Constituents	Concentration
Alkaloids	+
Tannins	+++
Saponins	_
Flavonoids	_
Cardiac Glycosides	
• Lieberman's	+
• Salkowski	+++
• Keller kiliani	+++
Anthraquinones	
Free hydroxyanthraquinones	_
Combined Anthraquinones	+++
Phlobatanins	-

Key: +++ = High concentration
++ = Moderate concentration
+ = Low concentration
= Not detected

Figure 2: Phytochemical properties of Hibiscus Rosa-Sinensis L. Leaf

Effect of *Hibiscus rosa sinensis* leaves extract on body weight (g) of mice

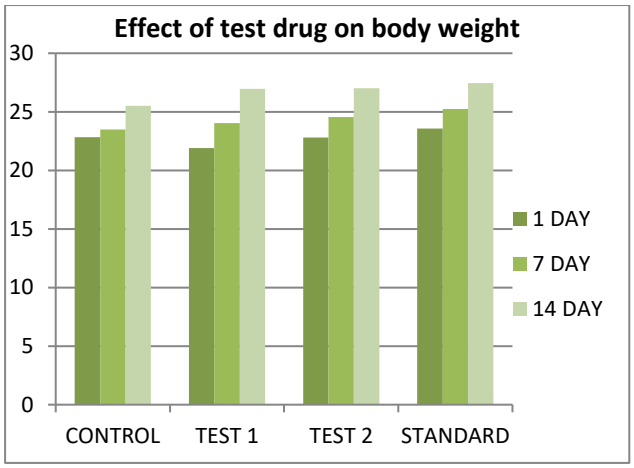

Figure 3: Graph showing effect of *Hibiscus rosa sinensis* leaves extract on body weight (g) of mice.

Effect of *Hibiscus rosa sinensis* leaves extract on Feed intake (g) of mice.

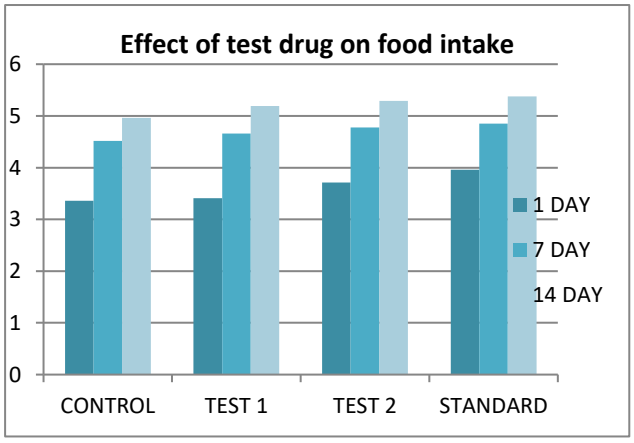

Figure 4: Effect of *Hibiscus rosa sinensis* leaves extract on Feed intake (g) of mice.

Effect of *Hibiscus rosa sinensis* leaves extract on Water intake (ml) of mice.

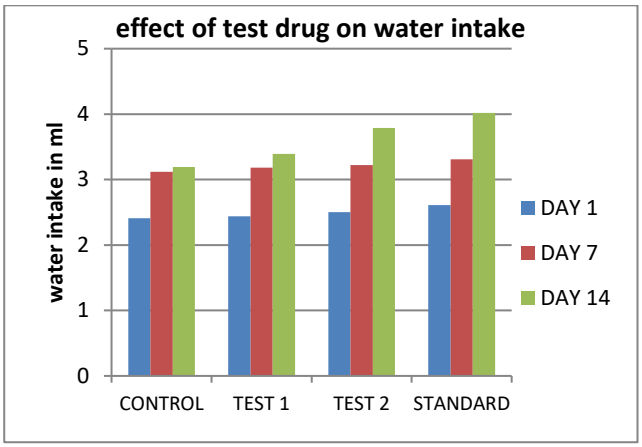

Figure 5: Effect of *Hibiscus rosa sinensis* leaves extract on Water intake (ml) of mice.

EVALUATION OF ANTIDEPRESSANT EFFECT OF *HIBISCUS ROSA SINENSIS* LEAVES ETHANOLIC EXTRACTS IN LOCOMOTION, TST & FST MODELS

Effect of *Hibiscus rosa sinensis* extract on locomotors activity of mice

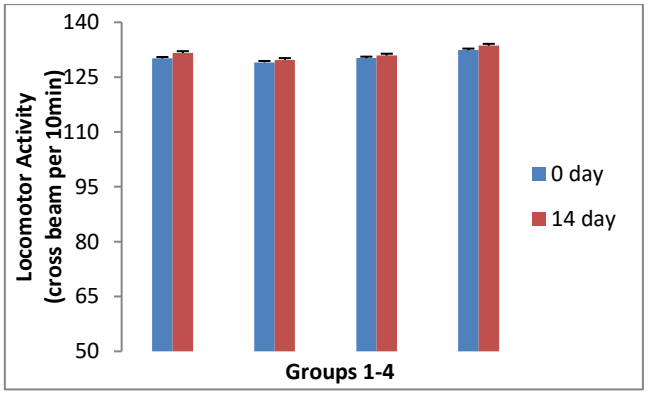

Figure 6: Graph Showing Effect of *Hibiscus rosa sinensis* extract on locomotors activity of mice.

Tail Suspension Test (TST)

When compared with the control group. All values represent = Mean ± SEM, n= 6 in each group

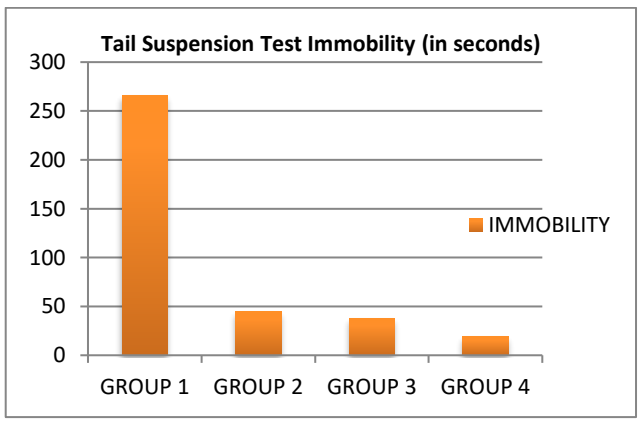

Figure 7: Graphical representation of effects of different doses of *Hibiscus rosa sinensis* leaves ethanolic extract on antidepressant effect as compared to standard (Fluoxetine)

Forced swimming test:

Ethanolic extract of *Hibiscus rosa sinensis* leaves (EEHRSL)

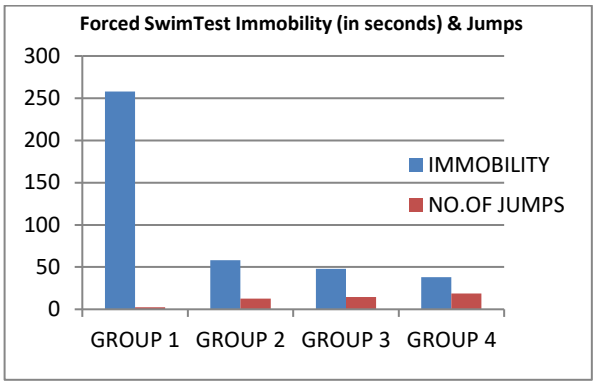

Figure 8: Graphical representation of effects of different doses of Ethanolic extract of *Hibiscus rosa sinensis* leaves on antidepressant effect as compared to standard (Fluoxetine).

Effect of Ethanolic extract of *Hibiscus rosa sinensis* leaves on plasma corticosterone levels (CORT)

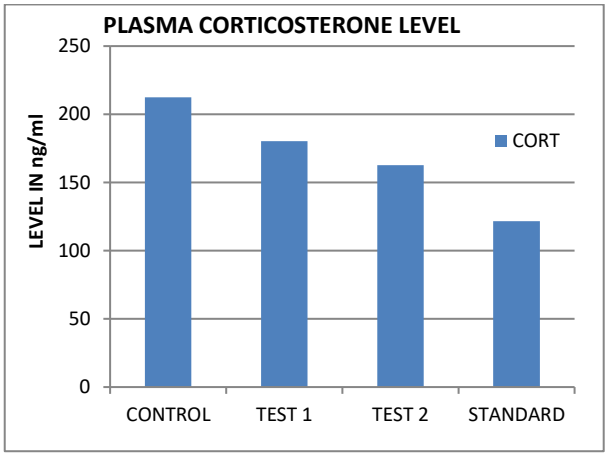

Figure 9: Effect of *Hibiscus rosa sinensis* extract on plasma corticosterone levels

It is recommended that each laboratory establish its own normal range since corticosterone levels can vary due to handling and sampling techniques.

DISCUSSION

Progress in unraveling the neuro-chemical mechanism is, as in so many areas of psychopharmacology, limited by the lack of good animal models of the clinical condition. All the following parameters were performed with suitable time interval to prevent unwanted stress in animals.

In Acute oral toxicity study, The median lethal dose (LD_{50}) of Ethanolic extract of *Hibiscus rosa sinensis* leaves (EEHRSL) was determined in accordance with the Organization for Economic Co-operation and Development (OECD, 425) guidelines using five mice which were fasted overnight before dosing with ethanolic extract of *EEHRSL* separately at maximum dose level up to 1000 mg/kg orally starting from dose of 100, 250, 500 mg/kg. One mouse was initially dosed and food was further withheld for 4 h. It was observed for the first 24 h and then for 14 d for signs of toxicity

(changes in mucous membranes, skin, fur and eyes, circulatory, respiratory, somato-motor activity and behaviour pattern) and mortality. It has been observed that no change in behavioural responses and observation shows any acute oral toxicity. The remaining four mice were also dosed and observed for 2 weeks. Thereafter, the LD_{50} was estimated.

The mice of *Hibiscus rosa sinensis* leaves extract (200 & 400 mg/kg/p.o) treated group showed significantly (p<0.05) increased in body weight as compared to the control group. Treatment with FXT (10 mg/kg i.p) the body weight significantly increased as compared to normal group.

The mice of *Hibiscus rosa sinensis* leaves extract (200 & 400 mg/kg/p.o) treated group showed significantly (p<0.05) increased in feed intake as compared to the control group. Treatment with FXT (10 mg/kg i.p)the feed intake significantly (p<0.05) increased as compared to control group.

The mice of *Hibiscus rosa sinensis* leaves extract (300 &600 mg/kg/p.o) treated group showed significantly (p<0.05) increased in body water intake as compared to the control group. Treatment with FXT (10 mg/kg i.p) the water intake significantly (p<0.05) increased as compared to control group.

The mice of *Hibiscus rosa sinensis* extract (200 & 400 mg/kg/p.o) treated group showed no significantly (p<0.05) difference in locomotors activity as compared to control group. Treatment with FXT (10 mg/kg p.o.)also showed no significant change as compared to control group.

There are no major differences or significant in different groups of Animals treated with FXT (Standard) EEHRSL (low and high dose) and control group.

The mice treated group of *Hibiscus rosa sinensis* extract (200 & 400 mg/kg/p.o.) showed increased plasma corticosterone level (p<0.05) as compared to normal control mice. However pretreatment) FXT (10 mg/kg i.p)14 days showed significant increased (p<0.05) in plasma corticosterone significantly.

Hibiscus rosa sinensis, which belongs to Malvaceae family, has been widely used as a traditional remedial plant in China and several tropical countries. All of its parts have been used in the treatment of fever, inflammation, bacterial infections, and even as contraceptive agent. Flavonoids, tannins, terpenoids, saponins, and alkaloids are the main phytochemicals as they are present in different extracts, and are more likely responsible for their biological activities.

The present study aims to investigate the effect of Hibiscus rosa sinensis in environmental induces depression in Swiss albino mice. In this study we administered Hibiscus rosa sinensis extract will be administered p.o to mice daily in two different doses (200 & 400 mg/kg/p.o) for 14 days. Hibiscus rosa sinensis reported to increase in body weight, feed and water intake. Water intake and feed intake efficacy in Hibiscus rosa sinensis groups was significantly increased within one week of treatment. In our study, treatment with Hibiscus rosa sinensis increased in the food and water intake as compared to control group. Thus, in the light of above discussed results we conclude that Hibiscus rosa sinensis extract may have anti-depressant potential. However further studies are very much required to enlighten the mode of action and other effects.

After selection of Hibiscus rosa sinensis, acute oral toxicity was detected with ethanolic extracts (EEHRSL) having dose (100, 250, 500, 1000 mg/kg) via oral route , shows no change in behavioral responses and observation shows no acute oral toxicity. Hence depending upon it, Dose was selected 200 mg/kg & 400 mg/kg for our experimental work. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by ethanol provided more consistent activity compared to those extracted by water. The results of antidepressant activity of plant Hibiscus rosa sinensis against the investigated FST and TST parameters are shown in table. The higher dose extract produced more effect comparative to lower extract in both parameters. This might have resulted from the lack percentage of the active constituents in ethanol extract showed some degree of antidepressant activity. Further trials using solvents of various polarities will explore the effects of solvent composition on extract efficacy.[17]

Hibiscus rosa sinensis (200 mg/kg & 400 mg/kg) ethanol extract was evaluated in albino mice in Tail Suspension test (TST) and forced swim test (FST) The effects of fluoxetine (FXT; 10 mg/kg) were also being assessed.

In **TST (Tail suspension test),** Group - Control 0.9% w/v sodium chloride Normal saline (10 ml/Kg) or (1ml / 100gm, p.o.) administration have shown their immobility is insignificant. When Ethanolic extract of Hibiscus rosa sinensis (200 mg/kg) orally administered, have shown immobility is significant. When Ethanolic extract of Hibiscus rosa sinensis (400 mg/kg) orally administered, have shown immobility is significant. When standard dose of fluoxetine (10 mg/kg) was orally administered, have shown immobility is more significant.

In **FST (Forced swimming test),** Group - Control 0.9% w/v sodium chloride Normal saline (10 ml/Kg) or (1ml / 100gm, p.o.) administration have shown their immobility and number of jumps is insignificant.

When Ethanolic extract of Hibiscus rosa sinensis (200 mg/kg) orally administered, have shown immobility and number of jumps is significant. When Ethanolic extract of Hibiscus rosa sinensis (400 mg/kg) orally administered, have shown immobility and number of jumps is significant. When standard dose of fluoxetine (10 mg/kg) was orally administered, have shown immobility and number of jumps is more significant.

The Ethanolic extract of Hibiscus rosa sinensis 400 mg/kg showed the most remarkable activity. This plant can be further subjected to isolation of the therapeutic

Antidepressant compound and carry out further pharmacological evaluation.

SUMMARY AND CONCLUSION

Hibiscus rosa sinensis is a rich source of nutrients as well as phytochemicals. Richness in vital components make it a wholesome tree with several medicinal and health properties. It has been used traditionally to cure many diseases and is also an important ingredient of Ayurveda herbs. Animal based studies have proved its effectiveness to prevent and cure diseases and hence strengthened its claim of herbal medicine.

Hibiscus rosa sinensis contains several phytoconstituents like β-sitosteryl-D-glucoside, vitamin K, n-octacosanol, kaempeferol, quercetin, and myricetin. The plant has been studied for their various pharmacological activities like antibacterial, antifungal, anticonvulsant, immunomodulatory, antioxidant, hypoglycemic, hypolipidemic, anthelmintics, and wound healing activities. Scientists from divergent fields are investigating plants anew with an eye to their Antimicrobial usefulness. A sense of urgency accompanies the search as the pace of species extinction continues.

Deficiencies of certain vitamins, minerals and amino acids causes anxiety, nervousness, irritability, general weakness, headache, apprehension, depression, sleep difficulty, fatigue, and chronic stress. The Hibiscus rosa sinensis is a rich source of vitamins, minerals and amino acids, which may be helpful in antistress activities and its antioxidant activity is already proved (the plant with antioxidant property generally shows antistress activity). Therefore, we have undertaken this particular study.

From our investigation of screening of Hibiscus rosa sinensis, the results obtained confirm the therapeutic potency of plant used in traditional medicine. In addition, these results form a good basis for selection of candidate plant species for further Phytochemical and pharmacological investigation.

In conclusion, Hibiscus rosa sinensis extracts possess a broad spectrum of activity against a panel of factors responsible for the most common psycosis diseases. These promissory extracts open the possibility of finding new clinically effective antidepressant compounds. The ethanolic extracts of Hibiscus rosa sinensis, investigated individually for Antidpressant activity by FST and TST method at the dose level of 200 & 400mg/ml. The ethanolic extract higher dose of Hibiscus rosa sinensis showed considerably high activities than lower dose. These results were compared with standard antidepressant fluoxetine. But the exact active components of the extract that showed this effect were not isolated. In conclusion, although active components were not isolated, but antidepressant active plant principles were observed in the both extracts.

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Authors Contributions: All the authors have contributed equally.

Source of Support: Nil

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Ethics approval: Research protocol is duly approved by the Ministry of Environment and Forests, Government of India's Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (Reg. No. 2011/PO/Re/S/18/CPCSEA, registration date: 1/5/2018),

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