

Antianxiety Activity Evaluation of *Hibiscus Sabdariffa* Linn. Calyces

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ABSTRACT

Background: Anxiety is one of the most commonly prevailing psychiatric and behavioural disorders. Evolutionarily, it is regarded as a healthy emotion or behavioural repertoire, which is the outcome of a stressful situation, thereby, helping an individual to cope with a possible risk or a novel situation by increasing awareness and responsiveness. Benzodiazepines, barbiturates, SRIs etc., are used as first line drugs for the management of anxiety, but due to myriad of adverse effects associated with these conventional drugs, inclination of patients towards complementary and alternative medicines (CAMs) has tremendously risen. CAM interventions mainly include herbal remedies. Different parts of *Hibiscus sabdariffa* have been used traditionally for treating several ailments including mental disorders.

Purpose: Keeping in mind the traditional use of *H. sabdariffa* calyces, the present study was designed to evaluate their antianxiety potential.

Methods: Petroleum ether (60-80°C), chloroform, ethanol, and water extracts were prepared using successive Soxhlet extraction and evaluated using elevated plus-maze model.

Results: Among all the extracts, ethanol extract showed significant antianxiety activity at a dose of 400 mg/kg.

Conclusion: Thus, the study establishes antianxiety activity of *H. sabdariffa* calyces and also validates their traditional use.



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1. Introduction

Anxiety is one of the most common affective disorder which is prevalent worldwide. According to WHO reports, 3.6 % of the global population suffers from anxiety (WHO, 2017). Anxiety is characterized as a frequent negative emotion accompanied with excessive worry, muscle tension, restlessness, impaired concentration, sleep disturbances and fatigue. Numerous classes of synthetic drugs like barbiturates, benzodiazepines, serotonin reuptake inhibitors, monoamine oxidase inhibitors and phenothiazines are widely employed for the treatment of anxiety (Chatterjee *et al.*, 2011). Though effective to a great extent, these drugs are associated with numerous unwanted side effects. As a result, the inclination of people towards herbal remedies is tremendously increasing as these both efficacious and have fewer side effects.

Hibiscus sabdariffa Linn., commonly known as Lal Ambari, is a plant found in the tropical and subtropical regions of the world (Christine *et al.*, 2017). The plant is an annual, erect, bushy sub-shrub growing up to 8 ft. The leaves are 8-15 cm long, arranged alternately on the stems. The

flowers are white to yellow with fleshy red calyx at the base (Mak *et al.*, 2013). Various parts of the plant have been used in traditional system of medicine as diuretic, diaphoretic, choleric, laxative, antifungal, antibacterial and antitussive and also for the management of nervous disorders (Qi *et al.*, 2005). Despite widespread medicinal use of the plant, no significant reports pertaining to its antianxiety activity properties are available. Therefore, the present study was planned to evaluate antianxiety activity of *H. sabdariffa* calyces.

2. Material and Methods

2.1. Plant Material

Dried calyces of *H. sabdariffa* were purchased from Earth Expo Company, Gujarat, India.

2.2. Chemicals and Reagents

Solvents used include petroleum ether 60-80°C (Merck India Ltd., Mumbai), chloroform (Thermo Fisher

Scientific India Pvt., Ltd., Mumbai), ethanol (Panipat Sugar Mill, D-Unit, Panipat), and distilled water prepared in our laboratory. Diazepam (Java Pharmaceuticals, Gurugram) was used as standard antianxiety drug.

2.3. Preparation of Extracts

Coarsely powdered calyces of *H. sabdariffa* (1kg) were Soxhlet extracted successively with pet ether, chloroform, and ethanol. The marc was finally boiled with distilled water to prepare the water extract. Exhaustive extraction with each of the solvent was ensured. The extracts were dried using Eylei N 1100 rotary vacuum evaporator and were preserved in a vacuum desiccator containing anhydrous silica gel blue.

2.4. Experimental Animals

Laccai mice (either sex), housed at the Central Animal House, Panjab University, were allowed standard pellet diet (Ashirwadi, Chandigarh) and water *adi libitum*. Groups of 6 mice (20-30 g) were used in all sets of experiments. The animals were fasted for 18 h before use. Approval (PU/IAEC/S/16/112) from the Institutional Animal Ethical Committee of Panjab University, Chandigarh, was taken before carrying out biological studies.

2.5. Preparation of Doses

Tween 80 (5%) in aqueous carboxymethyl cellulose (CMC 0.5% w/w) was used as a vehicle for preparing the suspension of extracts and standard drug. Doses of test extracts were prepared by suspending appropriate quantity in the vehicle so as to administer it to mice in volume ranging between 0.20 and 0.30 mL per oral route.

2.6. Acute Toxicity Study

Acute toxicity study of ethanol extracts of *H. sabdariffa* calyces were carried out on mice as per the OECD 423 guidelines (OECD, 2001). After 12 h of fasting, different groups of mice were administered single oral dose (500, 1000, and 2000 mg/kg) of the extracts. Immediately after dosing, animals were observed for signs of toxicity during the first 0.5, 1, 2, 4, 8, and 12 h and at every 24 h for 14 days. Behavioural parameters, tremors, lethargy, death, amount of water, and feed taken were observed.

2.7. Antianxiety Activity

Antianxiety activity was evaluated using elevated plus-maze (EPM) model (Lister, 1990; Pellow *et al.*, 1985). The apparatus consisted of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 12 cm) having an open roof.

The apparatus was kept elevated (25 cm) from the floor for evaluating the anxiolytic behavior. Doses were administered orally using tuberculin syringe fitted with an oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the EPM 60 min after the administration of the vehicle, diazepam (2 mg/kg), or the test extract (100, 200, 400 mg/kg). Each mouse was placed at the center of EPM with its head facing toward the open arm. During 5 min duration of the experiment, behavior of the mouse was recorded as (a) the number of entries into the open arms and (b) mean time spent by the mouse in open arms.

2.8. Statistical Analysis

Results have been expressed as mean ± standard error mean. The significant difference among the groups was assessed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The results were considered statistically significant at $P < 0.001$. Statistical analysis was performed using the Graph Pad Prism 5.

3. Results and Discussion

3.1. Yield of the Extract

Yield of pet ether, chloroform, ethanol, and water extracts of *H. sabdariffa* calyces was observed to be 3.43, 6.72, 33.30, and 8.14% w/w, respectively.

3.2. Acute Toxicity Studies

No toxic effects were observed up to a dose of 2000 mg/kg of extracts.

3.3. Antianxiety Activity of Extracts

Despite the traditional use of *H. sabdariffa* calyces for treating nervous disorders, there is an absence of scientific reports on the evaluation of its antianxiety effects. EPM model is considered to be etiologically similar to the anxiety observed clinically in human beings (Lister, 1990). An anxiolytic agent increases both the frequency of entries and the time spent in open arms of the EPM.

Administration of diazepam (2 mg/kg) significantly increased the number of entries and the time spent in the open arms compared to the control group. Among the four extracts of *H. sabdariffa* calyces, only the ethanol extract exhibited statistically significant ($p < 0.001$) antianxiety activity at a dose of 400 mg/kg (Table 1). Besides this, a dose-dependent decrease in antianxiety was observed, which might be due to mild sedation at higher dose.

Table 1: Antianxiety activity profile of various extracts of *H. sabdariffa* calyces using EPM

Treatment	Dose (mg/kg)	Number of entries in open arms (Mean \pm SEM)	Time (sec) spent in open arms (Mean \pm SEM)
Vehicle	0.25 ml	2.8 \pm 0.40 ^b	5.5 \pm 0.42 ^b
Diazepam	2	7.6 \pm 0.42 ^a	16.3 \pm 0.49 ^a
Petroleum ether extract	100	3.1 \pm 0.47 ^b	6.0 \pm 0.68 ^b
	200	3.0 \pm 0.51 ^b	5.8 \pm 0.74 ^b
	400	3.1 \pm 0.40 ^b	6.0 \pm 0.85 ^b
Chloroform extract	100	4.5 \pm 0.42 ^b	7.8 \pm 0.60 ^b
	200	4.1 \pm 0.60 ^b	9.1 \pm 0.47 ^b
	400	4.0 \pm 0.57 ^b	8.1 \pm 0.60 ^b
Ethanol extract	100	4.8 \pm 0.60 ^b	10.0 \pm 0.36 ^b
	200	5.1 \pm 0.54 ^b	13.1 \pm 0.30 ^b
	400	7.3 \pm 0.71 ^a	15.8 \pm 0.60 ^a
Water extract	600	5.0 \pm 0.73 ^b	10.1 \pm 0.60 ^b
	100	3.5 \pm 0.84 ^b	8.1 \pm 0.47 ^b
	200	3.5 \pm 0.67 ^b	7.1 \pm 0.47 ^b
	400	3.8 \pm 0.47 ^b	7.0 \pm 0.57 ^b

*n=6. p<0.001. ^a: vs control; ^b: vs diazepam. One-way ANOVA followed by Tukey's multiple comparison test.

Conclusion

Results of the present study indicate that the ethanol extract of the *H. sabdariffa* calyces has a significant antianxiety activity at a dose of 400 mg/kg. Also, the study validates the traditional use of *H. sabdariffa* calyces.

Author Contribution

Dr Anupam: Study conception and design.

Dr Gulsheen: Study Investigation, Analysis and interpretation of results and manuscript writing.

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Conflict of interest

Nil

Declaration

It is an original data and has neither been sent elsewhere nor published anywhere.

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