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RESEARCH ARTICLE

Evaluation of antiepileptic activity on *Hibiscus sabdariffa* leaves

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ABSTRACT

In Ayurveda literature of India, different parts of this plant have been recommended for various ailments such as hypertension, pyrexia, and liver disorder. It is traditionally used as antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, sedative, stomachic, and tonic.

Keywords: Antiepileptic, Hibiscus, Sabdariffa leaves

INTRODUCTION

Hibiscus sabdariffa (family: Malvaceae) consists of large number of cultivated species. This is an annual erect, bushy, and herbaceous subshrub growing to 8 ft (2.4 m) in height bearing auxiliary white flowers with a reddish center at the base of the staminal column and fleshy and bright red fruits. The red anthocyanin pigments present in their calyces are used as food coloring agents.[1] In Ayurvedic literature of India, different parts of this plant have been recommended for various ailments such as hypertension, pyrexia, and liver disorders. It is traditionally used as antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, sedative, stomachic, and tonic. It is also reported to be used in the treatment of paralysis, epilepsy, convulsions, and spasm.[2] The phytochemical review highlights presence of vitamins such as riboflavin, niacin, and ascorbic acid. Other constituents such as β-carotene, anisaldehyde, arachidonic acid, citric acid, malic acid, tartaric acid glycinebetaine, trigonelline, anthocyanins, cyanidin-3-rutinoside, delphinidin, delphinidin-3-glucoxyloside, hibiscus acid, delphinidin-3-sambubioside, and cyanidin-3-sambubioside are also reported.[3] Most of the CNS disorders such as epilepsy, insomnia, and pain are treated by modern system of medicine. Although the effect of medicine is immediate, there are number of associated side effects which are alarming when used for long duration of time.[4] Thus to avoid these side effects such as dizziness, double vision and stomach upset, and lethargy that are associated with the prolonged anti-epileptic medication, many herbal drugs such as *Taxus wallichiana*, *Nardostachys jatamans, Scutellaria baicalensi, Cestrum nocturnum, Bacopa monnieri*, and *H. sabdariffa* Linn. are being used in the form of extracts, formulations, or as an isolated compounds. Thus, the plant *H. sabdariffa* due to its natural abundance and therapeutic importance deserves additional evaluation with respect to its antiepileptic potential.

MATERIALS AND METHODS

Experimental methodology

Identification and collection of plant material

The leaves of *H. sabdariffa* were collected from local market of Gudlavalleru and Gudivada. The collected leaves washed thoroughly in distilled water to remove contaminates and dried under room temperature.

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Preparation of extract using maceration method

The dried leaves of *H. sabdariffa* were coarsely powdered and separately subjected to extraction by maceration in methanol at room temperature and with occasional shaking for 4 days. The macerate and the filtrate were dried at low temperature (40–50°C) under vacuums. The extracts were stored in airtight containers until further use.[5-7]

Phytochemical analysis

Phytochemical analysis of extract was carried out for the presence of alkaloids, glycoside, flavonoids, tannins, saponins, steroids, terpenoids, etc., by different methods.

Tests for alkaloids

1. Dragendorff's test (potassium bismuth iodide): To the extract, 1 ml of Dragendorff's reagent was added. An orange-red precipitate indicates the presence of alkaloid.
2. Wagner's test (solution of iodine in potassium iodide): To the extract, Wagner's reagent was added. Reddish-brown precipitate indicates the presence of alkaloid.
3. Mayer's test (potassium mercuric iodide): To the extract, 1 or 2 ml of Mayer's reagent was added. A dull white precipitate indicates the presence of alkaloid.
4. Hager's test (saturated solution of picric acid): To the extract, 3 ml of Hager's reagent was added. Yellow precipitate indicates the presence of alkaloid.

Tests for carbohydrates

1. Molisch's test: To the extract, 1 ml of α-naphthol solution was added and conc. sulfuric acid was added along the sides of test tube. Purple or reddish-violet color at the junction between the two liquids indicates the presence of carbohydrates
2. Fehling's test: To the extract, equal quantities of Fehling's solution A and B were added. On heating gently, a brick red precipitate indicates the presence of carbohydrates
3. Benedict's test: To 5 ml of Benedict's reagent, eight drops of solution under test were added and mixed, and the mixture was boiled vigorously for 2 min and cooled. A red precipitate indicates the presence of carbohydrates.

Tests for proteins

1. Biuret test: To the extract, 1 ml of 40% sodium hydroxide and two drops of 1% copper sulfate solutions were added. A violet color indicates the presence of proteins
2. Xanthoproteic test: To the extract, 1 ml of concentrated nitric acid was added, a white precipitate formed, it was boiled and cooled. Then, 20% of sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids
3. Lead acetate test: To the extract, 1 ml of lead acetate solution was added. A white precipitate indicates the presence of proteins.

Test for amino acids ninhydrin test

Two drops of freshly prepared 0.2% ninhydrin reagent were added to the extract and heated. Development of blue color indicates the presence of proteins, peptides, or amino acids.

Tests for steroids and sterols

1. Liebermann–Burchard test: The test extract was dissolved in 2 ml of chloroform in a dry test tube. Ten drops of acetic anhydride and two drops of concentrated sulfuric acid were added. The solution becomes red, then blue, and finally bluish-green in color indicating the presence of steroids
2. Salkowaski test: The extract was dissolved in chloroform and an equal volume of conc. sulfuric acid was added. Bluish-red to cherry red color is observed in chloroform layer, whereas the acid layer assumes marked green fluorescence indicating the presence of steroids.

Tests for glycosides

1. Legal test: The extract was dissolved in pyridine and sodium nitroprusside solution added to it and made alkaline. Pink-red or red color indicates the presence of glycosides

Table 1: Phytochemical analysis

S. No.	Phytochemical	Test	Results
1.	Flavonoids	NaOH test	+
2.	Tannins	$FeCl_3$	+
3.	Saponins	Frothing test	+
4.	Glycosides	Keller-Killiani test	+
5.	Steroids	Liebermann–Burchard test	_
6.	Alkaloids	Dragendorff's test	+
7.	Phenols	Ferric chloride test	+
8.	Triterpenoids	Thionyl chloride	+
8.	Carbohydrates	Molisch's test	+
9.	Proteins	Biuret test	+
10.	Fixed oils	Saponification test	_

Values expressed as mean±SEM

Table 2: Extensor phase and time taken for recovery (MES-induced convulsions)

S. No.	Group	Dose (mg/kg)	Extensor phase (s)	Time taken for recovery (s)
1.	Control	-	14.46±0.82	105.4±1.109
2.	Standard	5	0.00±0.00	66.80±1.16
3.	Ethanol extract (HS)	100	4.82±0.34	97.44±0.87
4.	Ethanol extract (HS)	200	8.85 ± 0.55	89.85 ± 1.96

Table 3: Onset of convulsion and time taken for recovery (PTZ-induced convulsion)

S. No.	Group	Dose (mg/kg)	Onset of convulsions (s)	Time taken by recovery (s)
1.	Control+PTZ	70	81±	1455.5±67.03
2.	Standard+PTZ	5	0±0	0±0
3.	Ethanol extract (HS)+PTZ	100	121±3.38	760±81.8
4.	Ethanol extract (HS) +PTZ	200	135.83 ± 10.19	708 ± 12.16

Value expressed as mean±SEM

2. Baljet test: To the extract, sodium picrate solution was added. Yellow to orange color indicates the presence of glycosides
3. Borntrager's test: Few milliliters of dilute sulfuric acid were added to the test solution. Boiled, filtered, and extracted the filtrate with ether or chloroform. The organic layer was separated and treated with ammonia. Pink, red, or violet color indicate the presence of glycosides
4. Keller-Killiani test: The sample was dissolved in acetic acid containing trace of ferric chloride and transferred to the surface of conc. sulfuric acid. At the junction, reddish-brown color was formed, which gradually becomes blue indicating the presence of glycosides.

Test for flavonoids Shinoda test

To the extract, magnesium turnings were added, followed by the addition of conc. hydrochloric acid. A red color indicates the presence of flavonoids.

Tests for tannins

1. To the extract, ferric chloride was added. Dark blue or greenish-black color indicates the presence of tannins
2. To the extract, potassium dichromate solution was added. A precipitate indicates the presence of tannins.[8]

Test for triterpenoids

In the test tube, two or three granules of tin were added and dissolved in 2 ml of thionyl chloride solution. Then, test solution was added. Production of pink color indicates the presence of triterpenoids.

Tests for fixed oils

1. Spot test: A small quantity of extract was pressed between two filter papers. Oil stains on paper indicate the presence of fixed oils
2. Saponification test: To the extract, few drops of 0.5 N alcoholic potassium hydroxide were added along with a drop of phenolphthalein. The mixture was heated on a water bath for 1–2 h. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils.

Experimental animals

Male albino Wistar rats weighing 130–150 g were procured and housed in animal house of V.V. Institute of Pharmaceutical Sciences. Animal house was well maintained under standard hygienic conditions, at a temperature (22 ± 20°C) and room humidity (60 ± 10%) with 12 h day and night cycle, with food and water *ad libitum*. All pharmacological works were carried out as per

CPCSEA norms, after obtaining approval from the Institutional Animal Ethical Committee of V.V. Institute of Pharmaceutical Sciences, Gudlavalleru, India.

Maximal electroshock (MES)-induced convulsion method

Each group comprised four animals.

- Group 1: Control (electroconvulsive shock 150 mA, 0.2 s, using ear electrode)
- Group II: Standard (phenytoin 5 mg/kg i.p. + electroconvulsive shock 150 mA, 0.2 s, using ear electrode)
- Group III: Ethanol extract (100 mg/kg i.p + electroconvulsive shock 150 mA, 0.2 s, using ear electrode)
- Group IV: Ethanol extract (200 mg/kg i.p + electroconvulsive shock 150 mA, 0.2 s, using ear electrode).

A total of 16 rats were divided into four groups. Group 1 received 1 ml/rat of saline, Group II received 5 mg/kg of phenytoin intraperitoneal (i.p) route of administration, and Groups III and IV received 100 and 200 mg/kg, respectively (i.p). The saline and standard reference drug were administered 45 min before induction of seizure, whereas the test extracts of *H. sabdariffa* were administered 1 h before induction of seizure. To induce convulsions in the control and drug-treated animals, the maximal (tonic hind limb extension) electroshock seizure (MES) test with supramaximal stimulation was carried out through transauricular copper electrodes (introduced bilaterally into the ears) with the apparatus (Inco Electroconvulsiometer model# 100- 3), using a fixed current 150 mA in rats for 0.2 s. The tonic extension of the hind limbs (extensor phase) and mortality was recorded.[9]

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Phytochemical analysis of the ethanol extract of *H. sabdariffa* leaves showed the presence of compounds glycosides, flavonoids, saponins, alkaloids, tannins, and triterpenoid [Table 1].

Antiepileptic activity by maximal electric shock-induced seizures:

Each group of animals values the extensor phase of seizures and time taken for recovery note done on Table 2.

Each group of animals values the onset of convulsions and time taken for recovery [Table 3].

CONCLUSION

Based on the results obtained in the present study revealed that ethanolic extract of *H. sabdariffa* leaves contains flavonoids, tannins, alkaloids, glycosides triterpenoids, phenols, carbohydrates, and proteins. Epileptic seizures induced by MES and PTZ methods. In the present investigation, HS (200 mg/kg) demonstrated a significant protection from PTZ- and MES-induced seizures, whereas HS (100 mg/kg) exhibited comparatively mild protective effect. Hence, it could be deduced that *H. sabdariffa* may be modulating the duration and frequency of opening the GABA-mediated chloride channels, leading to the protection from epileptic seizures.

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