Research Article

Phytochemical Properties and Antioxidant Activity of *Hibiscus sabdariffa* Linn

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ABSTRACT

Hibiscus sabdariffa is one of the important constitutents among the widely herbal formulations. *Hibiscus sabdariffa* linn. having family – Malvaceae was reported to have wide ethno medicinal use. Leaves are orally used as a stomachic and externally as an emollient. They are mainly used as diuretic and anthelmentic. Its decoction is useful for high blood pressure and cough. Main contents of herbal plant *Hibiscus sabdariffa* are steroid, hibiscin, tannins, carbohydrates, flavonoid are known to exhibit a range of biological activities like antiinflammatory, antihypercholesterolemic, antihyperlipidemic, anti viral, anthelmintic and hypotensive and properties due to their free radical scavenging ability. First of all, Phytochemical screening of Hibiscus sabdariffa leaves, for extract (Water extract, 95% ethanol extract and ethyl acetate fraction) was performed then the extracts were dried and were subjected to qualitative chemical tests. Fibre content & Phenol content of different extracts were determined. Anti oxidant activity was also determined by DPPH method.

Keywords: *Hibiscus sabdariffa*, Malvaceae, *Diphenly Picyl hydrazyl* method, Anti oxidant activity.

INTRODUCTION

Antioxidants are supposed to reduce the risk of cancer and other diseases by helping the body to get-rid of oxygen freeradicals which are thought to contribute to cancer development by damaging the DNA. Many plants and their extracts are rich source of agents such as anti-oxidants which can prevent the occurrence of cancer by reducing freeradical induced cell damage. Because of the vast utility of the anti-oxidants, in treatment of various diseases including cancer as discussed earlier, we were interested to screen the aqueous, 95% ethanol, extract and ethyl acetate fraction of leaves of *Hibiscus sabdariffa Linn,* belonging to family Malvaceae, for its antioxidant potentials. Despite a long tradition of use, no work has been carried out to justify its traditional claims, specially, anti oxidant properties. The plant is an erect annual herb with a reddish cylindrical stem nearly glabrous, Leaves are simple, having petiole, blade 3-5 lobed or parted, the lobes serrated. Flowers are solitary, axially, 5-7 cm in diameter; Calyx is thick, red prominently 10-nerved; petals 5, Red, stamens are numerous. The fruit is capsule, ovoid1-2 cm long, A native of tropical Africa or Asia. It is cultivated in warm countries, particularly in Philippines, Malaya and Indonesia. In India, it is grown in Punjab, U.P., Bihar, Bengal, Bombay, Mysore, Andhra and Chennai as a common crop. Chemical constituent of herbal plant *Hibiscus sabdariffa* are organic acids, tannins, carbohydrates, flavonoid, steroid, hibiscus acid, hibiscin, hibiscitrin.

MATERIAL AND METHODS

Hibiscus sabdariffa plant leaves were collected from the medicinal plant garden of Baba Isher Singh College of Pharmacy, Gagra, Moga.

Chemicals Used

Ethyl acetate, 95 % ethanol, chloroform water were used in the preparation of extracts in phytochemical screening and DPPH was used to study anti oxidant activity.

Extractive values

These help in evaluating the constituents of crude drug, which cannot be determined by any other means. The amount of an extract that a drug yields in a particular solvent is often an approximate measure of the amount of certain constituents that the drug contains.

Table 1: Extractive value of Hibiscus sabdariffa leaves

s. No.	Plant material	Extractive values (%w/v)	
		Alcohol soluble	Water soluble
1	Hisbiscus sabdariffa	13.05	15.92

Ash values

This parameter can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components.

Table 2: Ash value of Hibiscus sabdariffa leaves

S. No.	Types of Ash	Ash Value in %w/w
1.	Total ash	7.50
2.	Acid insoluble ash	1.06
3.	Water soluble ash	2.12
4.	Sulphated ash	8.25

Estimation of crude fibre

Crude fibre consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60% to 80% of the cellulose and 4% to 6% of the lignin. The crude fibre content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration quality and quantity.

Calculation

$$\% \text{ Crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample}} \times 100$$

The fibre content of leaves of *Hibiscus sabdariffa* was tabulated in table 3.

Table 3: Fibre content of leaves of *Hibiscus sabdariffa*

S. No.	Plant	fibre content %w/w
1	Leaves of Hibiscus sabdariffa	9.96

PHYTOCHEMICAL SCREENING

Leaves of *Hibiscus sabdariffa* were powdered and Soxhlet extracted with petroleum ether, chloroform, methanol and water. It was filtered, concentrated, and dried on water bath at 50-60 C. Exhaustive extraction with each of the solvents was ensured. The three dried extracts were preserved in vacuum desiccators. All the extracts were dissolved in respective solvents and were screened for different classes of phytoconstituents.

1. Aqueous extract
2. 95% ethanol extract
3. Ethyl acetate fraction

Table 4: The colour, consistency and yield of the extracts and fraction

Name	Colour	Consistency	Yield (%w/w)
Aqueous extract	Dark brown	Powder	16.5
95% ethanol extract	Dark green	Sticky Powder	14.1
Ethyl acetate fraction	Light green	Powder	4.03

Table 5: Phytochemical screening of herbal plant *Hibiscus sabdariffa* leaves extracts

S. No.	Tests	Aqueous extract	95% Ethanol extract	Ethyl acetate fraction
1	Alkaloids	-	-	-
2	Carbohydrates	+	+	+
3	Proteins	+	+	+
4	Amino Acid	+	+	+
5	Glycoside	+	+	+
6	Steroids and Sterols	-	+	+
7	Anthraquinones	-	-	-
8	Flavonoids	+	+	+
9	Tannins and Phenol compounds	+	+	+
10	Triterpenoids	+	+	+
11	Saponin Test	-	-	-
12	Fixed oils	-	-	-

(+) Present, (-) Absent

Fluorescence analysis of powder

The fluorescence character of powdered leaves of *Hibiscus sabdariffa* was studied both in day light and UV light.

Table 6: Fluorescence Analysis of powder

S. No.	Drug	UV Light	Visible Light
1	1N NaoH	Yellow	Yellow
2	Ammonia	Greenish yellow	Green
3	1N HCl	Light brown	Green
4	50% HNO_3	Reddish brown	Green
5	Only powder	Green	Green

Phenol estimation

The phenol content in the different extracts of leaves of *Hibiscus sabdariffa* was estimated by colorimetric method.

Table 7: Phenol content of aqueous and 95% ethanol extract and ethyl acetate fraction

S. No.	Extracts and fraction	Phenol content (% w/w)
1	Aqueous extract	6.8
2	95% ethanol extract	17.83
3	Ethyl acetate fraction	43.11

ANTIOXIDANT STUDIES

Diphenyl Picryl hydrazyl (DPPH) Method

Chemical and reagents used

- *Diphenly Picryl hydrazyl solution* (DPPH, l00µM)[9]. 22mg of DPPH (2,2,Diphenyl-l-Picryi hydrazyl) was accurately weighed and dissolved in 100ml of methanol. From this stock solution, 10ml was taken and diluted to 100ml using methanol to obtain l00µM DPPH solution.
- *Dimethyl sulphoxide* (DMSO), distilled.
- *Methanol,* distilled.

Preparation of test solutions

21 mg of each of the aqueous extract, 95% ethanol extract and ethyl acetate fraction was dissolved in distilled Dimethyl sulfoxide (DMSO) to obtain a solution of 21 mg/ml concentration. Each of these solutions was serially diluted separately to obtain concentration of 1,000µg/ml to 0.015625µg/ml.

Preparation of standard solution

10mg of each of Ascorbic acid and Rutin were weighed separately and dissolved in 0.95ml of DMSO to get 10.5mg/ml concentrations. This solution was serially diluted with Dimethyl sulfoxide to get lower concentrations.

Method

The antioxidant activities of aqueous extract, 95% ethanol extract and ethyl acetate fraction was assessed on the basis of the radical scavenging effect of the stable DPPH free radical.

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used are 1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml and 15.625µg/ml. The plates were incubated at 37°C for 30 minutes and the absorbance of each solution was measured at 490nm, using ELISA reader against the corresponding test and standard blanks and the remaining DPPH was calculated. IC_{50} is the concentration of the sample required to scavange 50% of DPPH free radicals. IC_{50} values for each extract were calculated.

Table 8: Antioxidant activity by DPPH method

S. No.	Test compound	IC_{50} value (µg/ml)
1	Aqueous extract	94.16 ± 1.52
2	95% ethanol extract	46.13 ± 3.37
3	Ethyl acetate fraction	53.87 ± 2.56
4	Ascorbic acid	54.17 ± 1.27

RESULTS AND DISCUSSION

The Pharmacognostical studies of leaves of *Hibiscus sabdariffa* will be helpful in future for identification and authentication of the plant. It is clear that plant may have good phytoconstituents like flavonoids, steroids, carbohydrates which on auxiliary studies may give better results for the cure of different types of diseases. As previously mentioned plant showed good antioxidant activity as all the three extracts exhibited good DPPH radial scavenging activity with IC_{50} values ranging from 46.13 ± 0.37 to 94.16 ± 0.56 µg/ml. The plant may be used clinically better to explore other activities like antihyperlipidmic, antihypercholester olemic & antiviral activities for human society in future.

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