

PHARMACOGNOSTICAL, PRELIMINARY PHYTOCHEMICAL AND ANTIOXIDANT EVALUATION OF *HIBISCUS SABDARIFFA*, LINN.

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ABSTRACT

Hibiscus sabdariffa linn. having family – Malvaceae was reported to have wide ethno medicinal use. *Hibiscus sabdariffa* is one of the important constituents among the widely herbal formulations. Roots orally used as a stomachic and externally as an emollient. They are mainly used as diuretic and tonic. Its decoction is useful for high blood pressure and cough. Main contents of herbal plant *Hibiscus sabdariffa* are tannins, carbohydrates, flavonoid, steroid, hibiscin are known to exhibit a range of biological activities like antihypercholesterolemic, antihyperlipidemic, antiinflammatory, anthelmintic, hypotensive and anti viral 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) were performed then the extracts were dried and were subjected to qualitative chemical tests. The Ash value, Extractive value, 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 (DPPH) method, Anti oxidant activity.

INTRODUCTION

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Antioxidants are supposed to reduce the risk of cancer and other diseases by helping the body to get-rid of oxygen free-radicals 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 free-radical induced cell damage²². 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 anti-oxidant 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, ovoid 1-2 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

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

PHARMACOGNOSTICAL STUDIES

Morphological characters

Leaf

Leaves are simple, having petiole, blade 3-5 lobed or parted, the lobes serrated or obtusely toothed.

Flowers:

Flowers are solitary, axially, nearly sessile, 5-7 cm in diameter; consisting of epicalyx segments

B-12 distinct, lanceolate to linear adnate at base of the calyx; Calyx is thick, red and fleshy, cup like deeply parted, prominently 10-nerved; petals 5, Red, twice as long as calyx, stamens are numerous; the filaments united into a staminal column; style single, five branched near summit, stigma capitate.

Fruit:

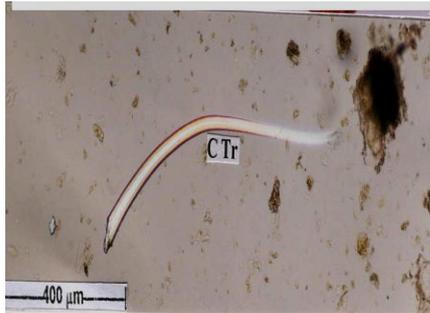
The fruit is capsule, ovoid, pointed, 1-2 cm long, shorter than calyx, having densely sharp and stiff hairs.

Microscopical characters

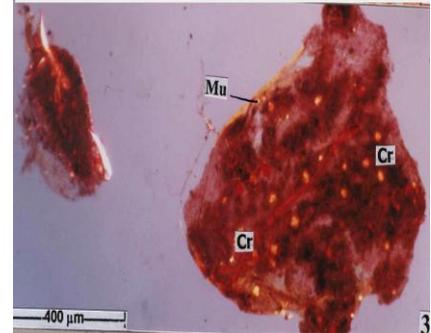
Powder microscopy

The powder of the leaf was examined under the microscope and some diagnostic features were observed.

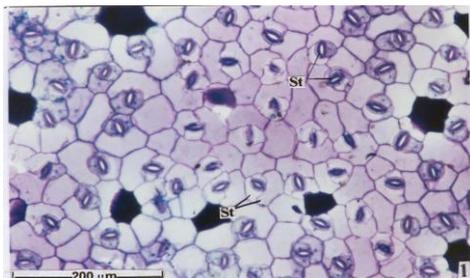
Unicellular, unbranched, thick walled pointed trichomes. The epidermal fragments have anomocytic or anisocytic stomata and straight anticlinal walls. Thick pieces of lamina and dense masses of mucilage were seen in the mesophyll tissue. Thick walled, pointed epidermal trichomes especially in the costal region were seen. Larger smaller druses of calcium oxalate crystals.



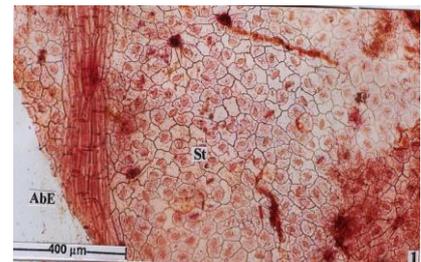
Covering Trichome



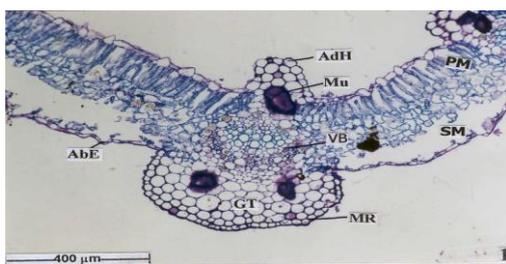
Mucilage and crystals



Abaxial epidermis with stomata



Abaxial epidermis with stomata



Leaf lateral Vein



Anisocytic stomata

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 no. 1. Extractive value of Hibiscus sabdariffa leaves

Sl. No.	Plant Material	Extractive Values (%w/v)	
		Alcohol soluble	Water soluble
1.	<i>Hibiscus sabdariffa</i>	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 CO₂ leaving behind the inorganic components.

Table no. 2. Ash value of Hibiscus sabdariffa leaves

Sl.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

Table no. 3. Fibre content of leaves of Hibiscus sabdariffa

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

Table no. 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 No. 5. Phytochemical screening of herbal plant Hibiscus sabdariffa leaves extracts

Sl. 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 no. 6. Fluorescence Analysis of powder

Sl. 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 no. 7. Phenol content of aqueous and 95% ethanol extract and ethyl acetate fraction

Sl.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

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

% 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 4.4.

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

ANTIOXIDANT STUDIES

Diphenyl Picryl hydrazyl (DPPH) Method

Chemical and reagents used:

Diphenyl Picryl hydrazyl solution (DPPH, 100 μ M)⁹. 22mg of DPPH (2,2-Diphenyl-1-Picryl 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 100 μ M 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 1,000 μ g/ml to 0.015625 μ g/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 200 μ l of DPPH solution, 10 μ l 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 1000 μ g/ml, 500 μ g/ml, 250 μ g/ml, 125 μ g/ml, 62.5 μ g/ml, 31.25 μ g/ml and 15.625 μ g/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. IC₅₀ is the concentration of the sample required to scavenge 50% of DPPH free radicals. IC₅₀ values for each extract were calculated.

Table no. 8. Antioxidant activity by DPPH method

SI. 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

. From the above mentioned data we can conclude that plant needed to be explored scientifically as more as possible, 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 radical scavenging activity with IC₅₀ 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, antihypercholesterolemic & antiviral activities for human society in future

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