



Pharmacological account of Hibiscus Sabdariffa Linn.

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ABSTRACT

Hibiscus sabdariffa Linn. is an annual herbaceous shrub, cultivated for its flowers although leaves and seeds have also been used in traditional medicine. The plant is reported to contain proteins, fats, carbohydrates, flavonoids, acids, minerals and vitamins. The plant has been reported to have antihypertensive, hepatoprotective, antihyperlipidemic, anticancer and antioxidant properties. The present paper is an overview on its pharmacological properties reported in the literature.

Keyword: Hibiscus sadariffa, Antihypertensive, Anticancer, Antioxidant, Flavonoids etc.

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INTRODUCTION

Hibiscus sabdariffa Linn. Is a shrub belonging to the family Malvaceae. It is thought of native to Asia (India to Malaysia) or Tropical Africa. The plant is widely grown in tropics like Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines as a home garden crop¹. In Sudan, it is a major crop of export especially in western part where it occupies second place area wise after pearl millet followed by Sesamum². Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which are generally extremely reactive and short lived. They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions. They are also found or generated through environmental pollution, cigarette smoke, automobile exhaust fumes, radiation, air-pollutants, pesticides etc.

There is increasing evidence to support the involvement of free radical reactions in several human diseases. In recent years it has become increasingly apparent that in man, free radicals play a role in a variety of normal regulatory systems. A majority of disease conditions like atherosclerosis, hypertension, ischaemic diseases, alzheimer's disease, Parkinson's disease, cancer and inflammatory conditions are being caused primarily due to the imbalance between pro-oxidant and antioxidant homeostasis³. The antibacterial effect of the leaves, stems and roots of Hibiscus sabdariffa was evaluated on bacterial strains like streptococcus aureus, Bacillus subtilis, Enterobacter aerogenes, and Escherichia coli⁴. The significant antibacterial activity of different extracts was compared with control where pure solvents were used instead of extracts⁵.

Anti-hyperlipidemic drugs can treat the primary disease but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of original disease rather than Hyperlipidemia⁶.

MATERIALS AND METHOD:

Pharmacological activity.

- Lipidemia
- Cholesterol associates with fat and protein and comes out of the liver as lipoprotein. There are several types of lipoproteins for the transport of fatty material in the body e.g.

chylomicrons, Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), Intermediate⁷

Antihyperlipedemic activity⁸

Animals:

Eighty female Sprague-Dawley albino rats (108± 4.2 g), 6-week old, were used. All animals were acclimated to the animal room for 1 week. The rats were housed in an animal room at 25 ± 2 °C under a 12 hour dark-light cycle (17:00-5:00 h) and relative humidity of 50-60%. Rats were divided into Eight feed groups:

- a) A basal diet (normocholesterolemic control rats; NC rats)
- b) Basal diet with 1% cholesterol (hypercholesterolemic rats; HC rats)

PLASMA BIOCHEMICAL ANALYSIS

At the end of the experimental period, overnight- fasted rats were anesthetized using ketamine (24 mg/kg b.w.) (Intramuscular injection) and sacrificed by decapitation. Blood samples were collected with a disposable plastic syringe into heparinized tubes.

Cholesterol esters are hydrolyzed to cholesterol and fatty acids. The cholesterol is oxidized by cholesterol oxidase to form cholesten-3-one and H₂O₂. This H₂O₂ oxidizes 4- amino antipyrine and phenol to a red colored compound, which can be measured at 505nm.

Reagents

a) Reagent 1

1. Buffer (50 mmol/L, pH 6.7)
2. Cholesterol oxidase ≥50 U/L
3. Cholesterol esterase ≥100 U/L
4. Peroxidase ≥3 IU/L
5. 4-amino antipyrine (0.4 mmol/L)

b) Reagent 2

1. Cholesterol standard (200 mg/dL)

Procedure

To 1.0ml of cholesterol reagent, 10µl of serum and standards were added separately. The contents were mixed well and incubated at 37°C for 10 minutes. The absorbance of the standard and sample was measured against reagent blank at 505nm within 60 minutes. The serum cholesterol is expressed as mg/dl.

Antihyperlipidemic activity

Effect of different parts of aqueous and ethanolic extracts of *H.Sabdariffa* Linn. on body weight in hypercholesterolemic rats.⁹

Feeding with 10% aqueous and ethanolic extracts of leaves, stem and roots of *Hibiscus sabdariffa* linn reduced the gain in body weight of experimental rats in both noncholesterolemic and hypercholesterolemic conditions.

Effect of different parts of aqueous and ethanolic extracts of *H. Sabdariffa* Linn. on lipid profile in hypercholesterolemic rats.

From the below Fig. no.1 it was found that rats treated with high cholesterol diet showed significant ($P<0.05$, $P<0.001$) increased in the level of TC (fig.7a), TG (fig.7b), PL (fig.7c), LDL (fig.7e) and VLDL (fig.7f) levels where as a significant ($P<0.001$) decreased in the level of HDL (fig.7d) as compared to control rats.

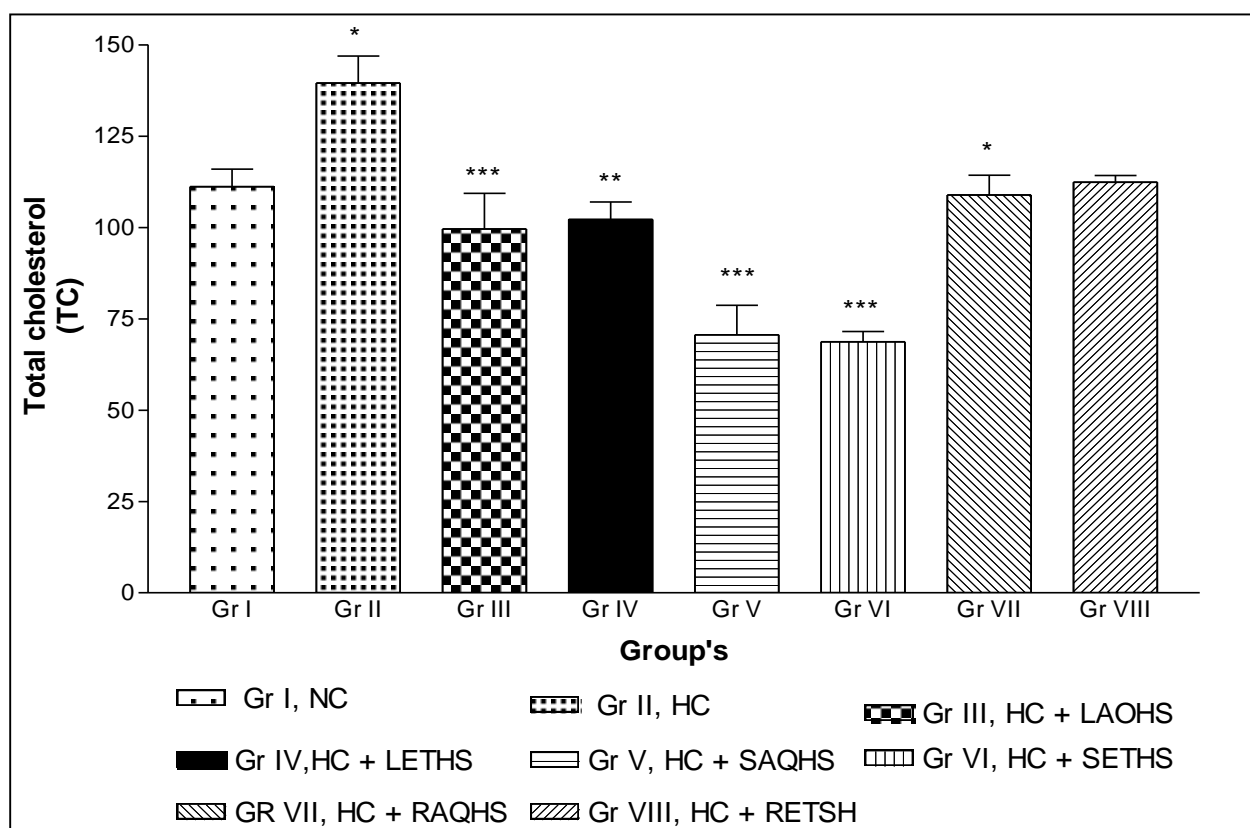


Figure 1: Effect of *H. sabdariffa* different parts extracts on total cholesterol level.

Antioxidant activity:

Fifty four Female Wistar albino rats (100-150 g) were used for this study. The animals were housed in large polypropylene cages in a temperature controlled room ($37^{\circ}\text{C}\pm 2^{\circ}\text{C}$) and provided with standardized pellet feed and clean drinking water *ad libitum*.¹¹

Experimental design:

After seven days of acclimatization, the rats were divided into nine groups (n=6). Treatment was done for 8 days as follows:

Group I: Control received the vehicle of 2% gum acacia (1ml/kg/day; p.o)

Group II: Toxic control received (CCl₄: olive oil (1:1); (0.7ml/kg.i.p) at every 72 h

After 24 h of the last dose, all the animals were then sacrificed and liver tissues were collected for the evaluation of *in vivo* antioxidant studies.

Estimation of lipid peroxidation (LPO), enzymic (CAT, Catalase) non-enzymic (GSH, Glutathion) antioxidant system:

Tissue supernatant preparation for LPO, CAT, GSH assay:

The livers were quickly removed, weighed and homogenized in phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at (1000 rpm, 15 min) to remove debris. The supernatant was used to assay the LPO, CAT, and GSH activities.

Determination of lipid peroxidation:

Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA). To 1 mL of supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) was added followed by, 0.5 mL of 0.8% TBA. The tubes were kept in a shaking water bath for 30 min at 80 °C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min.. The percentage inhibition of Lipid peroxidation was calculated using the equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where, A₀ is the absorbance of the control without extract and A₁ is the absorbance of the sample extract.

Antimicrobial activity of different extracts of *hibiscus sabdariffa* linn.¹²

Collection and identification of plant material.

Plant material:

The plant of *Hibiscus sabdariffa* linn was collected from the locations of Aurangabad (Maharashtra) region and was authenticated by Department of Botany, BAMU, Aurangabad. Plant material was preserved in Pharmacognosy department of Anuradha college of Pharmacy, Chikhali, Dist- Buldana, Maharashtra, India.

Extraction of Plant Material:

The plant of *Hibiscus sabdariffa* linn was separated into leaves, stem and roots and was air dried in shade. The powdered material was charged into the Soxhlet apparatus for defatting with petroleum ether followed by extraction with distilled water and 95% ethanol.

The powdered plant material was then macerated with distilled water and 95% (v/v) ethanol in a ratio of 1 to 4 (w/v) for both solvents. The suspensions of the samples were rotated on a shaker for 24 h at room temperature. Each suspension was then subjected for hot extraction by Soxhlet apparatus for 4 hrs.

Bacterial Strains

Microorganisms were obtained from the MGM, Biotechnology Department, Aurangabad, India. Amongst four microorganisms investigated, two Grampositive bacteria were *Staphylococcus aureus* and *Bacillus subtilis*, while remaining two Gram-negative bacteria were *Enterobacter aerogenes* and *Escherichia coli*

The antimicrobial assay was performed by agar cup method for both solvent extracts of leaves, stem and roots of *Hibiscus sabdariffa linn.*¹³

RESULTS AND DISCUSSION:

Effect of *Hibiscus sabdariffa linn* on the body weight in hypercholesterolemic rats.¹⁴

Feeding with 10% aqueous and ethanolic extracts of leaves, stem and roots of *Hibiscus sabdariffa linn* reduced the gain in body weight of experimental rats in both noncholesterolemic and hypercholesterolemic conditions. The percentage reduction in body weight is represented in the fifth column of table above.

Effect of *Hibiscus sabdariffa linn* on plasma lipid profiles in hypercholesterolemic rats.¹⁵

1. Plasma lipid profile concentrations in NC, HC, and HC + Aqueous or Ethanolic extract of Leaves or stem or roots of *Hibiscus sabdariffa linn*, fed rats after feeding for 6- week is presented in Table above.
2. Plasma TG, TC and PL in HC rats increased were found to be increased by 31.18%, 25.45% and 20.57%, respectively compared with levels in NC rats, Whereas these parameters decreased:
3. Moderately by 38.72%, 28.6% and 26.35%, respectively in HC+ LAQHS rats compared with HC rats.
4. Moderately by 37.3%, 26.72% and 26.2%, respectively in HC+ LETHS rats compared with HC rats.

Table 1: Effect of *Hibiscus sabdariffa linn* on the body weight in HC and treatment groups.¹⁰

Rat Groups	Initial body weight (g) (n=10)	Final body weight (g) (n=10)	Weight gained (g) (n=10)	% reduction in weight gain in NC and HC rats
NC	105 ± 5.3	203 ± 6.1	98 ± 7.6	-
HC	108 ± 4.9	254 ± 6.0	146 ± 4.6	-
HC+ LAQHS	107 ± 7.9	178 ± 4.9	71 ± 2.9	27.55 and 51.36
HC+ LETHS	110 ± 2.9	168 ± 3.6	58 ± 5.3	40.81 and 59.45
HC+ SAQHS	104 ± 3.3	114 ± 9.7	10 ± 4.2	89.79 and 93.15
HC+ SETHS	111 ± 6.4	129 ± 5.0	18 ± 2.3	81.83 and 87.67

sTable 2:Antibacterial activity of different parts of *H. sabdariffa* on different strain of microorganism.

Micro-Organisms used	Extract used Zone of Inhibition Results in mm	Control	LAQHS	LETHS	SAQHS	SETHS	RAQHS	RETHS
<i>Staphylococcus aureus</i>		22	09	10	21	23	19	20
<i>Bacillus subtilis</i>		19	10	10	20	18	19	20
<i>Enterobacter aerogenes</i>		24	21	23	19	18	21	21
<i>Escherichia coli</i>		22	19	19	24	20	20	21

Table 3: Effect of *Hibiscus sabdariffa linn* on the body weight in hypercholesterolemic rats.¹⁴

Rat Groups	Initial body weight (g) (n=10)	Final body weight (g) (n=10)	Weight gained (g) (n=10)	% reduction in weight gain in NC and HC rats
NC	105 ± 5.3	203 ± 6.1	98 ± 7.6	-
HC	108 ± 4.9	254 ± 6.0	146 ± 4.6	-
HC+ LAQHS	107 ± 7.9	178 ± 4.9	71 ± 2.9	27.55 and 51.36
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HC+ SAQHS	104 ± 3.3	114 ± 9.7	10 ± 4.2	89.79 and 93.15
HC+ SETHS	111 ± 6.4	129 ± 5.0	18 ± 2.3	81.83 and 87.67
HC+ RAQHS	103 ± 9.4	188 ± 4.1	85 ± 9.9	13.26 and 41.78
HC+ RETHS	105 ± 3.8	171 ± 0.8	86 ± 9.1	12.24 and 41.09

Table No.4 Effect of *Hibiscus sabdariffa linn* on plasma lipid profiles in hypercholesterolemic rats.¹⁵

Parameters mg/dL	NC N=10	HC N=10	HC+ LAQHS N=10	HC+ LETHS N=10	HC+ SAQHS N=10	HC+ SETHS N=10	HC+ RAQHS N=10	HC+ RETHS N=10
Plasma triglyceride (TG)	74.4 ± 5.3	97.6 ± 8.7	59.8 ± 1.9	61.2 ± 4.6	38.2 ± 6.4	31.9 ± 7.1	70.7 ± 3.8	71.4 ± 4.8
Total cholesterol (TC)	111.2 ± 4.8	139.5 ± 7.4	99.6 ± 9.7	102.2 ± 4.8	70.6 ± 8.1	68.7 ± 2.8	108.9 ± 5.4	112.4 ± 1.8
Phospholipid (PL)	164.3 ± 8.9	198.1 ± 7.7	145.9 ± 4.9	146.2 ± 8.1	89.9 ± 9.3	94.6 ± 6.4	157.9 ± 1.6	166.4 ± 2.9

Only the leaves aqueous and ethanolic extracts of *Hibiscus sabdariffa linn* showed significant antibacterial activity; this antibacterial activity was limited only towards the Gram Positive bacteria (*S. aureus* and *B. subtilis*) and was not seen with the Gram Negative bacteria cultures (*E. aerogenes* and *E. coli*).

Whereas percentage inhibitions of LPO, CAT and GSH were non-significant with the groups of both aqueous and ethanolic extracts of both stem and roots of *Hibiscus sabdariffa linn*.¹⁶

The antioxidant activity or the inhibition of the generation of free radical is important in the protection against CCl₄-induced liver lesion. Treatment with leaves extracts (Aqueous and ethanolic) of *Hibiscus sabdariffa linn* and Silymarin significantly reversed these changes.¹⁷

CONCLUSION:

The reported pharmacological studies support its traditional uses and may prove to be useful for clinical evaluation and development of commercial drugs. Introduction and commercial cultivation of its varieties in India is also recommended.

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