



Potential Antioxidant, Hypoglycemic and Hypolipidemic Effect of Leaves of *Hibiscus platanifolius* Linn

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ABSTRACT

The aim of present study was to investigate antioxidant, hypoglycemic and hypolipidemic effect of leaves of *Hibiscus platanifolius* Linn on rats and estimation of total phenolic and flavonoids content in ethanolic extract. The study of hypoglycemic activity involves induction of diabetes to rats of all the groups using alloxan (150 mg/kg i.p) followed by subsequent treatment with ethanol and aqueous hot extracts at two different doses each. Glibenclamide (0.5 mg/kg per day for one week) used as a reference standard. Blood glucose, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol and total proteins were estimated from the serum by using standard kits. The antioxidant activity was accessed by reducing power and hydrogen peroxide scavenging assay. The total phenolic and flavonoids content was determined by Folin-Ciocalteu and AlCl_3 reagent method, respectively. The biochemical parameters were increased in all diabetes rats; there parameters were decreased by the administration of ethanolic and aqueous hot extracts of leaves of *Hibiscus platanifolius* at dose of 100 mg and 150 mg/kg and are nearly similar to normal levels. *Hibiscus platanifolius* exhibited its scavenging effect in concentration dependent manner on hydrogen peroxide and property of metal chelating and reducing power. The content of flavonoids was $57 \pm 5.4 \text{ mg g}^{-1}$ and that of phenolic was $289.5 \pm 5 \text{ mg g}^{-1}$. From this study it has been concluded that the ethanolic and aqueous hot extracts of leaves of *Hibiscus platanifolius* having good antioxidant, hypoglycemic and hypolipidemic effect.

Keywords: *Hibiscus platanifolius* Linn, antioxidant, hypoglycemic, hypolipidemic, Rats.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural source, many based on their use in traditional medicines or phytomedicines. Various medicinal plants have been used for years in daily life to treat disease all over the world. According to world health organization (WHO), medicinal plants are the best source to obtain a variety of newer herbal drugs. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry some antibiotics have become almost obsolete because of drug resistant and consequently new drugs must be sought, for which herbal treatment is one possible way to treat diseases caused by multi drug resistant bacteria.

The use of plant extracts and phytochemicals, with known

antioxidant, anti diabetic and antihyperlipidemic properties may be of immense importance in therapeutic treatment. [1-2]

In the past few years, a number of studies have been conducted in different countries to prove such efficiency.

Hibiscus platanifolius Linn (Malvaceae) known as Maple-leaved mallow is an important medicinal plant. It is an evergreen tree, growing up to 10 m tall. Leaves are alternate, simple, stipulate, petiolate and ovate to lanceolate, often with a toothed or lobed margin. Leaves are usually 3-5 lobed, 6-12 × 5-12 cm. Leaf are palmately veined, entire or various lobed. Flowers are pale pink, with each petal having a deep pink base. Flowers are about 3-5 inches across. Sepals are leathery, hairy. Stamen-column is 2-2.5cm long, pale pink. Maple leaved mallow is native to India. This is well known in Asia and Africa and is commonly used more widely as a natural source of food coloring and used to make wigs. [3-5]

The bark of *Hibiscus* contains strong bast fibers, these fibers used for making grass skirts. In ayurvedic literature of India, different parts of this plant have been recommended as remedy for various ailments like hyperlipidemic, diabetes, hypertension, liver disorders and as antidotes to poisoning chemicals. *Hibiscus* petal is used to stimulate thicker hair growth and to prevent premature graying, hair loss and scalp

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disorders. It acts as a natural emollient hair conditioner and can be used in hair washes and vinegar rinses for the hair. A number of active principles from this plant have been identified which include taraxeryl acetate, beta sitosterol, campestral, stigma sterol, cholesterol, ergosterol, lipids, citric, tartaric and oxalic acids, fructose, glucose, sucrose, flavonoids and flavonoid glycosides. However there is no exclusive report on the medicinal values of the leaves of *Hibiscus platanifolius* Linn. Hence the present investigation is designed to screen the phytochemical constituents and to evaluate the antioxidant, hypoglycemic and hypolipidemic potential on rats.

MATERIALS AND METHOD

Plant collection

The plant materials used in this study was collected from Talakona forest, near Tirupati, Chittoor district, Andhra Pradesh, during December 2010 and was authenticated by Dr. K. Madavachetty, S. V. University, Tirupati, Andhra Pradesh. The leaves were initially separated from the main plant body and rinsed with distilled water, dried under shade paper towel in laboratory and then homogenized into fine powder and stored in air tight bottles and were used for all the extraction process.

Drugs & Chemicals

Alloxan monohydrate was purchased from Sigma-Aldrich, USA. Glibenclamide was purchased from Cipla Ltd, India. All biochemical estimations were assayed by using kits from Span Diagnostics Ltd., India. All other bio-chemicals used in this experiment were purchased from Sigma-Aldrich, USA. All the chemicals were of analytical grade.

Experimental animals

Male Wistar rats of body wt. 180-200 g were obtained from central Animal House, Ratnam institute of Pharmacy, Nellore, Andhra Pradesh. They were housed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60-70%. They were kept under standard conditions of 12/12 h light and dark cycle. The animals were maintained with standard pellet diet (Chakan Mill, Pune) and water *ad libitum*. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. All studies were carried out using 6 animals in each group. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Preparation of extracts-Extraction of aqueous component Cold aqueous extraction

250 g of air dried powder of leaves of *Hibiscus platanifolius* was weighed and soaked separately in 500 ml cold water in a conical flask stopper with rubber cork and left undisturbed for 24 hours and then filtered off using sterile filter paper (What Man No: 1) into a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C. The extract was filtered with the help of muslin cloth and was subjected to centrifugation at 5000Xg for 5 minutes and the supernatant was obtained and stored at 4°C for further use.^[6]

Hot aqueous extract

250 g of air dried powder of leaves of *Hibiscus platanifolius* was weighed and soaked separately in 500 ml of hot water which was then boiled for 30 minutes and kept in a conical flask for 24 hours undisturbed and then filtered off using

sterile filter paper in a sterile conical flask and subjected to water bath evaporation, where the aqueous solvent was evaporated at its boiling temperature 100°C. The extract was filtered with the help of a muslin cloth and was subjected to centrifugation 5000Xg for 5 minutes and the supernatant was stored at 4°C for further use.^[7]

Solvent extraction-Ethanol extract

250 g of air dried powder of leaves of *Hibiscus platanifolius* was weighed and was placed in 500 ml of organic solvent (ethanol) in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 h. After 24 h it was filtered with the help of muslin cloth and centrifuged at 5000Xg for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume of one-fourth of the original volume, giving a concentration of 40 mg/0.1ml. It was stored at 40°C in air tight bottles for further studies.^[7]

Phytochemical screening

Phytochemical analysis of the both aqueous and solvent extract of *Hibiscus platanifolius* Linn was performed according to the methods of Harborn (1998) and Evans, C. W. (1989).^[8-9] By this analysis, the presence of several phytochemicals like flavonoids, tannins, saponins, sugars and glycosidase were tested.

Acute oral toxicity study

Acute oral toxicity studies were carried out according to OECD guideline AOT 425. Mice or rat fasted overnight prior to drug administration. Each animal received a single dose of plant extract (2000 mg/kg, p.o.). The animals were observed for mortality for 24 hours.^[10]

Statistical analysis

The results were expressed as mean ± SEM and statistically analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test.

Determination of total flavonoids contents

The content of total flavonoids was determined by aluminium chloride colorimetric method. The content of flavonoid was determined as quercetine equivalent. 10 mg/ml of ethanol extract in respective solvent (stock solution) was mixed with 2 ml aluminium chloride (2% w/v) in methanol and the solution was made up to 25 ml with methanolic solution of acetic acid (0.5% v/v) (probe solution PS). 1 ml of SS was made up to 25 ml with methanolic solution of acetic acid (contrast solution CS). The absorbance of PS and CS was measured at 420nm after 30 minutes using UV spectroscopy (Analytical-2060). The result expressed as percentage of total flavonoid content.

$$\%TFC = \frac{\text{Absorbance at 420} \times \text{dilution factor}}{\text{E value} \times \text{weight of extract in gms}} \times 100$$

Estimation of total phenolic content

The Folin-Ciocalteu reagent was used to determine the total phenolic content. The ethanol extract 1 ml (10 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent previously diluted with 7 ml deionized water. The solution was allowed to stand for 3 minutes, at 25°C before adding 0.2 ml of saturated sodium carbonate solution. The mixture was allowed to stand for another 120 minutes. The absorbance was measured at 725 nm. Gallic acid was used as standard for the calibration curve. The total phenolic content of the extract was calculated in terms of Gallic acid equivalent (GAE).^[11]

Experimental induction of diabetes in rats

The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of

150 mg/kg body weight. [12] After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycemia (i.e. with a blood glucose of 200-300 mg/dl) were used for the experiment.

Experimental design

The rats were divided into seven groups of six rats each after the induction of alloxan diabetes. [13-14]

Group-I	Normal control rats
Group-II	Diabetic control rats
Group-III	Diabetic rats received standard drug, Glibenclamide (0.5 mg/kg) for 28 days
Group-IV	Diabetics rats received aqueous hot extract (100 mg/kg) for 28 days
Group-V	Diabetics rats received aqueous hot extract (150 mg/kg) for 28 days
Group-VI	Diabetics rats received ethanol extract (100 mg/kg) for 28 days
Group-VII	Diabetics rats received ethanol extract (150 mg/kg) for 28 days

The blood samples were drawn on 7th, 14th, 21st and 28th day from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hours and the blood was centrifuged (2,500 rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol and total proteins. After 28 days the rats were sacrificed, pancreas and liver were harvested and immediately frozen in liquid nitrogen for biochemical estimation.

Biochemical parameters

Blood glucose, triglycerides, cholesterol, HDL-cholesterol, LDL-cholesterol and total proteins were estimated from the serum by using standard kits. [15-17]

Evaluation of *in-vitro* antioxidant activity

Reducing Power of *Hibiscus platanifolius*

The reducing power of nutraceutical herbs was determined according to the method of Oyaizu (1986). Extracts were prepared in different concentrations ranging from 1 to 5 mg/ml and 1 ml of each distilled water were mixed with phosphate buffer (2.5 ml, 2M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%); the mixture was incubated at 50°C for which was then centrifuged at 1500 rpm for 10 minutes. The upper layer of solution (2.5ml) was mixed with distilled water (2.5) and FeCl₃ (0.5ml, 0.1%), and the absorbance as measured at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power. The reducing power was expressed as AAE means that reducing power of 1 mg of sample is equivalent to reducing power of 1N mole ascorbic acid.

Hydrogen Peroxide scavenging Assay [18- 20]

The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100 mM) was prepared instead of 40mM in phosphate buffer saline of (pH 7.4) and various concentration of aqueous and ethanol extracts (100-1000µg/ml) were added to hydrogen peroxide solution (2 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for back ground subtraction. In case of control absorbance of hydrogen peroxide at 230 nm without sample extracts was

measured. The percentage inhibition activity was calculated using the formula

$$\% \text{ inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where, A₀ = Absorbance of the control (blank, without extract); A₁ = Absorbance in the presence of extract/standard taken as Gallic acid (10-100µg/ml)

The IC₅₀ of the extracts was compared with IC₅₀ of standard.

RESULTS AND DISCUSSION

Acute oral toxicity study

The results of acute toxicity studies indicate that none of the extracts studied showed any behavioral changes, toxic reaction and mortality even after 24 hours. The extract was found to be safe at the dose of 2000 mg/kg.

Preliminary Phytochemical screening

The results of preliminary phytochemical analysis of leaf extracts of *Hibiscus platanifolius* Linn showed the presence of carbohydrate, gums, protein, amino acid, steroids, alkaloids, tannins and flavonoids. All the extracts showed the absence of mucilage. The results are tabulated in Table 1.

Table 1: Preliminary phytochemical screening of *Hibiscus platanifolius* Linn

S. No	Tests	Cold Aqueous extract	Hot Aqueous extract	Ethanol extract
1	Carbohydrate	+	+	+
2	Gums	+	+	-
3	Mucilage	-	-	-
4	Proteins	+	+	+
5	Amino acids	+	+	-
6	Steroids	+	+	-
7	Glycosides	-	-	+
8	Tannins	+	+	+
9	Alkaloids	+	+	-
10	Flavonoids	+	+	+

(+) -- indicates presence ; (-) -- indicates absence

Table 2: Reducing power of alcoholic extract of *Hibiscus platanifolius*

Concentration (mg/ml)	Absorbance 700nm	
	Ascorbic acid	<i>Hibiscus platanifolius</i>
1	1.055	0.453
2	1.098	0.509
3	1.113	0.513
4	1.165	0.571
5	1.141	0.624

Table 3: Hydrogen Peroxide scavenging Assay

S. No	Concentration (µg/ml)	Hydrogen Peroxide scavenging Assay (% inhibitory concentration)	
		Aqueous hot extract	Ethanol extract
1	200	22	33
2	400	34	42
3	600	46	49
4	800	67	69
5	1000	78	76

Flavonoid and total phenol contents of the ethanol extracts

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process. [21-22] Phenolic compounds are a class of antioxidant agents which act as free radical terminators. [23] The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: $y = 0.0067x + 0.0132$, $r^2 = 0.999$). The flavonoid contents in the ethanol extract of *Hibiscus platanifolius* was found to be 57 ± 5.4 mg/g. The contents of total phenols that were measured by Folin-Ciocalteu reagent in terms of gallic acid

equivalent (standard curve equation: $y = 0.05x + 0.0545$, $r^2 = 0.9873$). The phenolic contents in the ethanolic extracts of *Hibiscus platanifolius* were found to be $289.5 \pm 5 \text{ mg/g}^{-1}$.

Reducing Power of *Hibiscus platanifolius* Linn

For the measurements of the reducing ability, the Fe^{3+} - Fe^{2+} transformation was investigated in the presence of *Hibiscus platanifolius*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Fig. 1 depicts the reductive effect *Hibiscus platanifolius*. Similar to the antioxidant activity, the reducing power of *Hibiscus platanifolius* increased with increasing dosage. The result shows that *Hibiscus platanifolius* consist of hydrophilic poly phenolic compounds that cause the greater reducing power. The results are shown in Table 2.

Hydrogen Peroxide scavenging Assay

As shown in Table 3, *Hibiscus platanifolius* also demonstrated hydrogen peroxide decomposition activity in a concentration dependent manner. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+} , and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. The decomposition of H_2O_2 by *Hibiscus platanifolius* may at least partly result from its antioxidant and free radical scavenging activity.

Anti-diabetic activity of *Hibiscus platanifolius*

In animals treated with alloxan (150 mg/kg i.p) to group-II, a significant increase in the serum glucose levels was observed on the 7th, 14th, 21st and 28th day, when compared to the normal group (Group-I). Group-III treated with standard drug (Glibenclamide-0.5 mg/kg p.o) showed a significant decrease in serum glucose levels on 7th, 14th, 21st and 28th day, when compared to the diabetic control group (Group-II). On administration of aqueous hot and ethanolic leaf extracts at different doses (100mg and 150mg/kg) i.e., Group-IV, V, VI

and VII, the blood glucose levels were decreased on 7th, 14th, 21st and 28th day, when compared to the control group (Group-II). So the result revealed that, the ethanolic and aqueous hot extract at dose of 150mg/kg has more significant anti-diabetic activity compared to ethanolic and aqueous hot extract at dose of 100 mg/kg. The results were tabulated in Table 4.

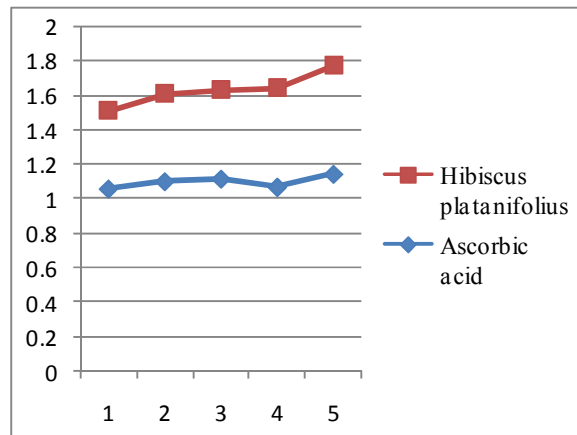


Fig. 1: Reducing power of *Hibiscus platanifolius* Linn and Ascorbic acid

Hypolipidemic activity

A significant decrease ($p < 0.001$) in the cholesterol, triglycerides levels were observed. Ethanol and aqueous hot extracts controlled the elevation of lipid profiles, cholesterol and triglycerides significantly in comparison with the standard control Glibenclamide. Hyperlipidemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and changes in lipoprotein composition. A marked increase in serum cholesterol and triglycerides levels was observed in diabetic rats. Treatment with Ethanol and aqueous hot extracts of *Hibiscus platanifolius* reduced the cholesterol and triglycerides level. The results were tabulated in Table 5. Ethanolic and aqueous hot extracts of *Hibiscus platanifolius* exhibited significant anti hyperglycemic and antihyperlipidemic activities in alloxan induced diabetic rats. Ethanolic extracts showed H_2O_2 radical scavenging activity and reducing power assay in rats.

Table 4: Effect of *Hibiscus platanifolius* on serum glucose levels in diabetic rats

Group	Treatment	Serum glucose (mg/dl) (Mean±SEM)				
		0 th day	7 th day	14 th day	21 st day	28 th day
I	Normal	62.40±0.08**	96.70±0.72**	86.80±0.72**	78.57±0.29**	61.70±0.02**
II	Alloxan control (150mg/kg)	224.70±0.44	194.00±0.04	207.80±0.81	211.60±0.41	232.30±0.56
III	Glibenclamide (0.5mg/kg)	176.6 ± 0.73**	113.75±0.13**	98.18±0.68**	92.00±0.07**	88.56±0.34
IV	Aqueous hot extract (100mg/kg)	216.20± 1.07**	102.68±0.28**	99.06±0.04**	93.07±0.93**	89.00±0.07**
V	Aqueous hot extract (150mg/kg)	226.00± 0.23**	78.87±0.57**	73.43±0.21**	78.22±0.84**	74.23±0.17**
VI	Ethanolic extract (100mg/kg)	228.80 ± 0.58**	91.62±0.73**	67.34±0.18**	62.12±0.52**	61.34±0.41**
VII	Ethanolic extract (150mg/kg)	225.80 ± 0.07**	90.61±0.46**	61.31±0.37**	60.09±0.14**	59.30±0.46**

Values were expressed as Mean ±SD; n=6. Statistical significance: (* $p < 0.01$, ** $p < 0.05$ and NS- Not Significant) One way ANOVA followed by Dunnett test

Table 5: Effect of *Hibiscus platanifolius* on lipid profile in alloxan induced diabetic rats

Treatment	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Total protein (g/dl)
Normal control	66.11± 0.27**	97.61±0.22**	6.09±0.02**
Alloxan control (150mg/kg)	177.6±0.18	184.68±0.16	3.17±0.16
Glibenclamide (0.5mg/kg)	71.24±0.19**	111.30±0.78**	5.28±0.31**
Aqueous hot extract (100mg/kg)	72.41±1.21**	115.64±1.06**	8.2±0.12
Aqueous hot extract (150mg/kg)	69.62±1.07**	108.31±0.11**	7.3±0.41**
Ethanolic extract (100mg/kg)	70.34±0.56**	117.52±0.27**	7.11±0.17**
Ethanolic extract (150mg/kg)	66.6±0.41**	107.15±0.31**	6.92±0.26**

Values were expressed as Mean ±SD; n=6. Statistical significance: (* $p < 0.01$, ** $p < 0.05$ and NS- Not Significant) One way ANOVA followed by Dunnett test

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