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PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIDIABETIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *HIBISCUS* LEAVES

Roli Shukla*

Department of Chemistry, Govt. MLB, Bhopal (M.P.)

***Correspondence Info:**

Dr. Roli Shukla
Professor, Depatment of Chemistry,
Govt. MLB College, Bhopal (M. P.)
Email:
k2pavni@gmail.com

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ABSTRACT

Nature has been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from natural source, many based on their use in traditional medicines or phytomedicines. Present investigation aim to access the *in vitro* antidiabetic activity of *Hibiscus* leaves extract against human pathogens. The content of total phenolic compounds (TPC) and total tannin content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the absorbance and Y is the tannic acid equivalent (GAE). Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y=0.040X + 0.009$, $R^2=0.999$, where X is the absorbance and Y is the quercetin equivalent (QE). The total phenolic and flavonoids content of the hydroalcoholic extracts were also determined. The results of the present study intigate that the Hydroalcoholic extract of *Hibiscus* of α-amylase showed maximum antidiabetic activity. Hence the extract may be useful as better therapeutic agent especially for the treatment of diabetes mellitus.

Key words: *Hibiscus,* Leaves, Phytochemical, Antidiabetic activity

INTRODUCTION:

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin , defective insulin action, or both (Sicree *et al.,* 2006). Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both (Shillitoe, 1988; Votey and Peters, 2004). Classification of diabetes mellitus is based on its aetiology and clinical presentation. As such, there are four types or classes of diabetes mellitus viz; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types (Sicree *et al.,* 2006). Type 1 diabetes is said to account for only a minority of the total burden of diabetes in a population although it is the major type of the diabetes in younger age groups at majority of well-to-do countries. The incidence of type 1 diabetes is increasing in both rich and poor countries. Furthermore, a shift towards type 1

diabetes occuring in children at earlier ages is imminent (Sicree *et al.,* 2006). 85 to 95% of all diabetes in high-income countries are of type 2 accounting for an even higher dominance in developing countries.

It is intimately associated with improper utilization of insulin by target cells and tissues. It is currently a common and serious health concern globally. According to WHO, (1994), this problem has been aggravated by rapid cultural and social dynamics, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioural patterns. Diabetes mellitus and lesser forms of glucose intolerance, particularly impaired glucose tolerance, can now be found in almost every population in the world and epidemiological evidence suggests that, without effective prevention and control programmes, diabetes will likely continue to increase globally (WHO, 1994).

MATERIAL AND METHOD

Following procedure was adopted for the preparation of methanol extracts from the shade dried and powdered herbs:

Defatting of Plant Material

Hibiscus (leaves) was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Dried powdered *Hibiscus* (leaves) has been extracted with hydroalcoholic solvent using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Investigation

The extract of L. parviflora was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins,
proteins, amino acids and flavonoids.

Total Phenolic content estimation (Hossain *et al.,* 2011)

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of each extract ethanolic and aqueous or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation (Hossain *et al.*, 2011)

Determination of total flavonoids content was based on aluminium chloride method, 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.

***In vitro* anti diabetic activity**

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100-1000µg/ml were prepared in methanol. 100 mg of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. 500 µl of this extract was for the estimation of enzyme inhibition. *In vitro* methods employed in antidiabetic studies. Inhibition of alpha amylase enzyme A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSIONS

The crude extracts so obtained after the maceration extraction process, extract was further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from different samples using Pet ether and hydroalcoholic as solvent is depicted in the table 1.

A small portion of the dried extracts were subjected to the phytochemical test using (Kokate, 1994) methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extract of all samples. Small amount of extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table 2.

The content of total phenolic compounds (TPC) and total tannin content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X+0.011$, $R^2= 0.998$, where X is the absorbance and Y is the tannic acid equivalent (GAE). Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: $Y=0.040X + 0.009$, $R^2=0.999$, where X

is the absorbance and Y is the quercetin equivalent (QE). The total phenolic and flavonoids content of the hydroalcoholic extracts were also determined. In both the extract more Flavanoidal content in comparison to Phenolic contents was found table 3.

Table No. 1: % Yield of *Hibiscus*

S. No.	solvent	% Yield (W/W)
1	Hydroalcoholic	3.4%

Table No. 5.2 Result of Phytochemical Screening of hydroalcoholic extract of *Hibiscus*

S.No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	-
2.	Glycosides	-
3.	Flavonoids	+
4.	Diterpenes	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	+
8.	Proteins	-
9.	Saponins	+

Table No. 3: Estimation of Total phenolics and Total flavonoids content

Extracts	Total phenolic content (mg/100mg of dried powder)	Total flavonoids Equvalent to Quercetin mg/ 100 mg of dried extract
Hibiscus	7.81	1.94

Table No. 4: Results of *In vitro* antidiabetic studies

S. No	Acorbose		Plant Extract	
	Conc.	% Inhibition	Conc.	% Inhibition
1	10	35.45	10	55.58
2	20	45.58	20	62.56
3	30	50.25	40	68.78
4	40	65.45	60	70.15
5	50	72.25	80	78.89
IC_{50} (µg/ml)	53.53		IC_{50}	92.25

CONCLUSION

The results of the present study intigate that the Hydroalcoholic extract of *Hibiscus* of α-amylase showed maximum antidiabetic activity. Hence the extract may be useful as better therapeutic agent especially for the treatment of diabetes mellitus.

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