

**EVALUATION OF ANTIDIABETIC POTENTIAL OF *CLERODENDRUM CHINENSE*****Alan Jacob^{1*}, Aswathi M.², Ajith Babu T. K.³**¹Associate Professor, Malik Deenar College of Pharmacy, Kasaragod.²Assistant Professor, KMCT Institute of Pharmaceutical Education and Research, Calicut.³Principal, Malik Deenar College of Pharmacy, Kasaragod.

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INTRODUCTION

Nature is usually a brilliant indicator of the common occurrence of coexistence. The foundation for treating human illnesses is natural items derived from plants, animals, and minerals. A demand for medicinal herbs exists, accompanied by a gradual rise in their acceptance.^[1] In addition, plants provide a rich nutritional supply of macromolecules, vitamins, and minerals that are essential for preserving bodily health. Many plants are found to have pharmacological effects because of their metabolites.^[2]

Primary metabolites and secondary metabolites are organic compounds found in plants. Glucose, starch, polysaccharides, protein, lipids, and nucleic acids are some of primary metabolites, which are organic substances that are good for the growth and development of body.^[2]

Secondary metabolites, such as flavonoids, alkaloids, terpenoids, saponins, tannins, glycosides, steroids, volatile oils, etc., are synthesised by plants. These secondary metabolites provide plants their medicinal efficacy and extensively used throughout the world in the treatment of numerous diseases. Worldwide, the practice of herbal based medicine is very common.^[2]

Natural products have been used for generations to treat common illnesses like allergies, colds, toothache and upset stomachs, and this trend is only growing. As a result, there has been a global movement toward the use of herbal remedies rather than synthetic ones, or a "Return to Nature" approach to illness prevention. Medicinal plants can be found in nature. Four billion people, or 80% of the global population, reportedly utilize herbal medicines for some form of primary healthcare, according to the World Health Organization (WHO).^[3]

Anti-diabetic activity

Diabetes Mellitus (DM) is a metabolic disease marked by persistently high blood sugar levels and varying degrees of impairment in the metabolism of proteins, fats and carbs.^[22] Diabetes mellitus (DM) can have many different causes, but at some time over the course of the disease, there will always be abnormalities in either insulin secretion, responsiveness, or both. Type-1 diabetes also known as immune mediated or idiopathic diabetes, and type-2 diabetes, formerly known as non-insulin dependent diabetes, account for the vast majority of patients with diabetes mellitus.^[4]

The treatment of diabetes mellitus, a dangerous metabolic disease, heavily relies on medicinal plants. It has been demonstrated that traditional plants have strong anti-diabetic properties without causing any unwanted side effects. 80% of individuals in developing nations use traditional botanicals as their first line of treatment, according to surveys. Traditional medicines are an integral part of primary healthcare in many developing countries.^[5]

Because medicinal plants are more easily accessible and less expensive than conventional pharmaceuticals, herbal therapy is being widely used to treat diabetes. When paired with insulin or other anti-diabetic drugs, this therapeutic approach can be used as an anti-diabetic treatment because of its minimal side effects. Numerous anti-diabetic plants have been found to have beneficial properties, such as antihypertensive, nephroprotective, and retinoprotective actions, in addition to their hypoglycemic action. These properties may help prevent the most common problems associated with diabetes.^[6]

MATERIALS AND METHODS

Plant collection and authentication

The stems of *Clerodendrum chinense* were collected from Payyannur. The botanist Dr. Biju P. from the Department of Botany at Government College, Kasaragod, verified the

authenticity of the plant sample. The plant materials were ground into a powder using a mechanical grinder, dried for a few days in the shade, and then sealed in an airtight container.

Successive solvent extraction^[7]

The dried powder of *Clerodendrum chinense* stems was extracted successively utilizing solvents with increasing polarity, such as petroleum ether, chloroform, ethyl acetate, methanol, and water. After weighing, moistening, and packing around 20g of dried powder in the Soxhlet apparatus, the powder was extracted using 500 ml of petroleum ether, chloroform, ethyl acetate, methanol, and water, respectively. The dried marc was used for the next extraction after each extraction. Following the filtering of each extract and the solvent's distillation, the dried extract was eventually obtained. Each extract's yield as a percentage was determined.

Qualitative phytochemical screening of ~~the~~ extracts^[8,9]

Chemical tests for glycosides

To find out whether glycosides were present, a little portion of the extract was hydrolysed for few hours on a water bath using diluted hydrochloric acid. The following tests were then performed on the hydrolysate.

Legal's Test

After evaporation, the residue (dry extract) was dissolved in a few millilitres of pyridine. Then it was turned alkaline by adding two millilitres of freshly manufactured sodium nitroprusside solution and sodium hydroxide solution. The creation of the colour pink-red was noted.

Baljet's test

A yellow-orange tint indicates the existence of cardiac glycosides in the extract, which when treated with one millilitre of sodium picrate solution.

Liebermann-Burchard's Test

Five millilitres of the hydrolysate were placed in a test tube, the residue was removed and placed in one millilitre of dry chloroform, and two millilitres of specifically distilled acetic anhydride were added. Finally, a few drops of strong sulphuric acid were added through the test tube's walls. Then, it was noticed that the upper portion had turned green, and the lower portion had developed a deep crimson colour that eventually turned blue and violet.

Borntrager's test

A small amount of the hydrolysate residue was combined with water and agitated with an equivalent volume of chloroform. After separating the chloroform layer, a diluted ammonia solution was added, thoroughly shaken, and it was observed if the ammoniacal layer had any pink hue.

Modified Borntrager's test

Ferric chloride and diluted HCl were applied to the residue to facilitate the oxidative hydrolysis of C-glycoside. Chloroform was then used to extract it. After separating the chloroform layer, a diluted ammonia solution was added and shook. For the colour pink, the ammoniacal layer was noticed.

Chemical test for phenolic compounds and tannins**Ferric chloride Test**

A tiny amount of the extract, which had been diluted with water, was subjected to a 5% diluted ferric chloride solution, and the presence of blue color was seen.

Gelatin Test

Water containing the dissolved extract was filtered; a 2% gelatin solution containing 10% sodium chloride, was added to the filtrate. Take note of the precipitate, which is milky white.

Lead acetate Test

A 10% lead acetate solution was used to treat the extract that had been dissolved in water. Take note of the precipitate, which is milky white.

Decolorization Test

The extract that had been dissolved in water was subjected to a solution of diluted potassium permanganate. Take note of the potassium permanganate's decolorization.

Chemical test for flavanones and flavonoids**Aqueous sodium hydroxide Test**

A few millilitres of the extract were mixed with an aqueous sodium hydroxide solution, and it was seen that the solution had a yellow tint.

- a. A tiny amount of the extract's alcoholic solution was applied to the filter paper. The filter paper's yellow colour was seen after it was subjected to ammonia fumes.

b. Shinoda's test

Add a small amount of concentrated HCl drop wise to the sample. The sample turned pink to crimson red with the presence of flavonoids indicated by the occasional green to blue colour appearing after few minutes.

Chemical test for Saponins**Foam or Froth Test**

In a graduated cylinder, a little amount of extract was diluted with 20 millilitres of distilled water. To check if any foam formed, the suspension was shaken for fifteen minutes.

Quantification of secondary metabolites**Estimation of Total Phenolic Content (Folin Cio-Calteau Method)^[10]****Preparation of reagents**

- a. Folin Cio-calteau reagent (2N): it was diluted to 1:10 ml with water.
- b. Sodium carbonate solution: 20% of sodium carbonate (anhydrous) made with 100 ml distilled water.

Preparation of standard graph of Tannic acid

In a 10ml Standard flask, 10mg of tannic acid was weighed and made up to 10ml with methanol. 1millilitre (1mg/ml) of the aforementioned solution was pipetted out, and 10ml of methanol were added to make a 100µg/ml tannic acid standard solution (stock solution). To obtain 2, 4, 6, 8, 10, 12, and 14 µg/ml solutions, respectively, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4-ml of the stock solution were pipetted out and made up to 10- ml with water. After 5minutes 5ml of Folin Cio-calteau reagent and 4ml of 20% sodium carbonate solution were added to the aforementioned solutions. After stirring, it was incubated for half an hour at room temperature. Using a Shimadzu UV-VIS spectrophotometer, the absorbance of the solutions was measured at 765 nm after 30 minutes.

Preparation of sample solution

Weigh and dissolve 10mg of the extracts in methanol and made up to 10ml with methanol. Pipette out 1ml from each extract solution and add 5ml of Folin Cio-calteau reagent. After 5 minutes add 4ml of 20% of sodium carbonate solution and let it sit at room-temperature for 30 minutes. Then measure the absorbance at 765nm and the values obtained were interpreted in the standard graph of tannic acid to get the milligram equivalents of tannic acid.

Estimation of Total Flavonoid Content^[11,12]

The colorimetric approach using Aluminium chloride was used for this. Concentrations 2, 4, 6, 8, and 10 µg/ml were made from the prepared stock solution. Every extract concentration was additionally manufactured. Add 4 millilitres of water and 0.3 millilitres of 5% sodium nitrate to each of these. 0.3 cc of a 10% aluminium chloride solution was added after 5 minutes. Add 2-ml of 1M sodium hydroxide and top up to 10-ml with distilled water after 6 minutes. After the solutions were combined, a UV-VIS spectrophotometer, was used to evaluate the absorbance at 510 nm against a blank solution (one in which aluminium chloride was not added). The standard graph of quercetin was used to compute the % of total flavonoid content.

Estimation of total glycoside content^[9]

A 100 ml volumetric flask was filled with 8 ml of plant extract, 60 ml of water, and 8 ml of 12.5% lead acetate. The mixture was then combined and filtered. To precipitate the excess Pb²⁺ ion, 50-ml of the filtrate was transferred into a second 100 ml flask, and 8 ml of 47% Na₂HPO₄ was added. This was combined with water to the desired volume. To get rid of extra lead phosphate, the mixture was filtered twice using the same filter paper. A clean Erlenmeyer flask was filled with 10 ml of pure filtrate, and then 10 ml of Baljet reagent was added. 10 milliliters of distilled water and 10 millilitres of Baljet reagent were used as a blank. For the full development of the colour, this was left to stand for an hour. At 495 nm, colorimetric measurement of the colour intensity was performed.

➤ ANTI-DIABETIC ACTIVITY^[13,14]**1. Alpha-amylase Inhibition Assay**

Plant extract was made at various concentrations using distilled water. A total of 500-µl of plant-extract and 500 µl of 0.02 M sodium phosphate-buffer (pH 6.9 with 0.006 M sodium chloride) containing alpha-amylase solution (0.5mg/ ml) were incubated for 10-minutes at 37°C. After pre-incubation, 500 µl of 1 % -starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube. This reaction mixture was incubated for 10 minutes at 37°C. 1ml of DNSA colour-reagent was added to stop the reaction. These test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. Finally, this reaction mixture was again diluted by adding 10ml distilled water following which absorbance was measured at 540nm. This procedure was

repeated for the standard drug Acarbose. The percentage alpha-amylase inhibition of both extract and standard drug was calculated by the following formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Calculation of IC₅₀ (50% inhibitory concentration)

From the graph, the drug's concentration (µg/ml) needed to scavenge 50% was determined. The IC₅₀ value was calculated for inhibitory concentration of both the sample and standard.

2. Alpha-glucosidase Inhibition Assay

To make the enzyme solution, dissolve 0.5 mg of α-glucosidase in 10 ml of pH 7 phosphate buffer with 20 mg of BSA. With phosphate buffer, it is further diluted to a ratio of 1:10. Following the preparation of various sample concentrations, 250µl of 20 mM p-nitrophenyl-α-D-glucopyranoside and 490µl of 100 mM phosphate buffer are mixed with 5µl of each solution or blank. After five minutes of pre-incubation at 37°C, 250µl of enzyme solution is added to initiate the reaction, and it is then incubated for fifteen minutes at 37°C. For the blank, 250µl of phosphate buffer is added in place of the enzyme. The amount of p-nitrophenol emitted is then quantified by measuring the absorbance of a sample at 400 nm against a blank, after which the reaction is halted by adding 1000µl of 200 mM sodium carbonate solution. The standard acarbose underwent the same process again. Using the following formula, the percentage inhibition of the standard and the extract was determined.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Calculation of IC₅₀ (50% inhibitory concentration)

From the graph, the drug's concentration (µg/ml) needed to scavenge 50% was determined. The IC₅₀ value was calculated for inhibitory concentration of both the sample and standard.

RESULTS

Plant collection and authentication

The plant *Clerodendrum chinense* (Lamiaceae) was collected from Payyannur in Kannur district. The plant material was taxonomically identified by the Botanist (Dr. Biju P, Dept. of Botany, Government College, Kasaragod) and dried under shade.

Successive solvent extraction of stems of *Clerodendrum chinense*

Using a Soxhlet apparatus, the coarse powder of the plant *Clerodendrum chinense* stems was extracted with solvents one by one. Following extraction, each extract's percentage yield was computed using the air-dried drug that was utilized in the investigation. The percentage yield and other characteristic features of the extract are tabulated below.

Table No. 1: Results showing the nature and percentage yield of the stem extracts of the plant *Clerodendrum chinense*.

Sl. No.	Solvent used for extraction	Colour	Consistency	Percentage yield (%w/w)
1	Petroleum ether	Yellowish green	Semi solid	3.86
2	Chloroform	Dark green	Semi solid	3.93
3	Ethyl acetate	Dark green	Semi solid	1.40
4	Methanol	Dark green	Semi solid	7.06
5	Water	Dark brown	Semi solid	14.86

Preliminary phytochemical screening

Table No 2: Results showing qualitative phytochemical screening of various stem extracts of the plant *Clerodendrum chinense*.

Sl. No	Qualitative test	Petroleum ether	Chloroform	Ethyl acetate	Methanol	Water
2	Glycosides					
	a. Legal's test	-	+	-	+	+
	b. Baljet test	-	+	-	+	+
	c. Liebermann Burchard test	-	+	-	++	+
	d. Bontrager's test	-	+	-	+	-
	e. Modified Bontrager's test	-	+	+	++	+
3	Phenolic compounds					
	a. Ferric chloride test	-	+	-	+	-
	b. Lead acetate test	+	++	-	+	+
4	c. Decolorization test	++	++	+	++	++
	Flavones & Flavonoids					
	a. Aqueous NaOH test	-	+	-	+	+
	b. Ammonia test	-	+	-	+	+
9	c. Shinoda's test	-	+	-	+	+
	Saponins					
9	a. Foam test	-	-	-	-	+

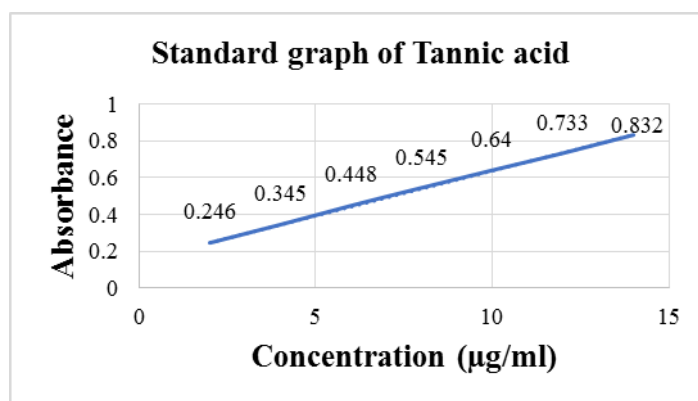
Quantification of secondary metabolites

Using the Folin Cio-Calteau technique, the total phenolic content present in each extract was determined. The standard graph was plotted using various concentrations of tannic acid. Then the total phenolic content in the extracts were determined and expressed in tannic acid equivalents.

Table No. 3: Results showing absorbance of Tannic acid at various concentrations.

Sl. No.	Concentrations (µg/ml)	Absorbance (at 765 nm)
1	2	0.246
2	4	0.345
3	6	0.448
4	8	0.545
5	10	0.640
6	12	0.733
7	14	0.832

A calibration curve of Tannic acid is plotted using absorbance on Y axis and concentration on X axis.

**Fig No.1: Calibration curve of Tannic acid.**

The absorbance values obtained for the extracts were recorded and the total phenolic content in the extracts were calculated in tannic acid equivalent and tabulated in the table given below.

Table No. 4: Result showing Total Phenolic content of different stem extracts of the plant *Clerodendrum chinense*.

Sl. No	Sample (Extract)	Absorbance (at 765nm)	Concentration from the graph (µg/ml)
1	Chloroform	0.399	5.07
2	Ethyl acetate	0.279	2.60
3	Methanol	0.470	6.53
4	Water	0.320	3.45

The mean absorbance value obtained for chloroform, ethyl acetate, methanol & water extract of stems of *Clerodendrum chinense* at a concentration of 100µg/ml of the extracts was found to be 0.399, 0.279, 0.470 and 0.320 respectively. The concentration of phenolic compound

present in chloroform, ethyl acetate, methanol and water extract was found to be 5.07, 2.60, 6.53 and 3.45µg/ml respectively. The total phenolic content present in the chloroform extract was found to be 507 tannic acid equivalents per gram, ethyl acetate extract 260 tannic acid equivalent per gram, methanol extract 653 tannic acid equivalent per gram and that of water extract 345 tannic acid equivalents per gram.

Estimation of total flavonoid content

Table No. 5: Results of total flavonoid content.

Sl. No.	Concentration (µg/ml)	Absorbance (510nm)
1	2	0.198
2	4	0.298
3	6	0.567
4	8	0.657
5	10	0.879
6	12	0.974
7	14	1.902

A calibration curve of Quercetin is plotted using absorbance on Y axis and concentration on X axis.

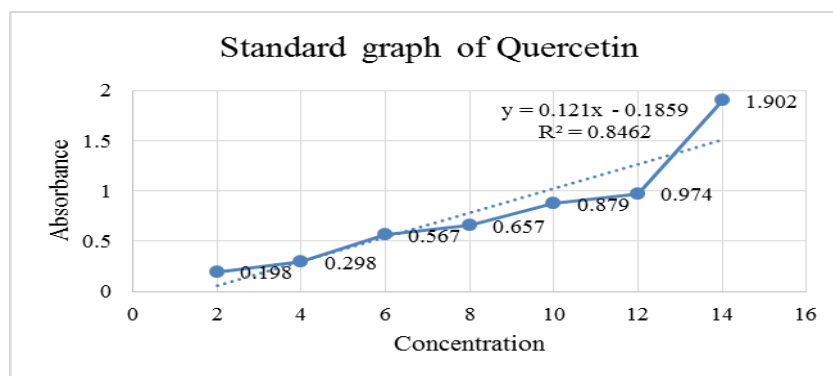


Fig No. 2: Standard graph of Quercetin for estimation of Total flavonoid content.

Table No. 6: Result showing total Flavonoid content of different stem extracts of the plant *Clerodendrum chinense*.

Si. No	Sample (Extract)	Absorbance (510 nm)	Concentration from the graph (µg/ml)
1	Chloroform	0.324	4.21
2	Ethyl acetate	0.198	3.17
3	Methanol	0.384	4.70
4	Water	0.258	3.66

The mean absorbance value obtained for chloroform, ethyl acetate, methanol & water extract of stems of *Clerodendrum chinense* at a concentration 100µg/ml of the extracts was found to be 0.324, 0.198, 0.384 and 0.258 respectively. The concentration of flavonoid compound present in chloroform, ethyl acetate, methanol and water extract was found to be 4.21, 3.17, 4.70 and 3.66µg/ml respectively. The total flavonoid content present in the chloroform extract was found to be 421 quercetin equivalent per gram, ethyl acetate extract 317 quercetin equivalent per gram, methanol extract 470 quercetin equivalent per gram and that of water extract 366 quercetin equivalent per gram.

Estimation of total glycoside content

Table No. 7: Results showing total glycoside content of stem of plant *Clerodendrum chinense*.

Weight of sample (g)	Absorbance at 495nm	Percentage of glycoside content (%w/w)	Average percentage (%w/w)
8	0.217	0.289	0.391
8	0.367	0.489	
8	0.297	0.396	

a) Invitro Antidiabetic activity

Alpha amylase inhibition assay

The alpha amylase inhibition assay was performed by various stem extracts of *Clerodendrum chinense*. The results are tabulated below.

Table No. 8: Results showing Invitro anti-diabetic activity of various stem extracts of the plant *Clerodendrum chinense* by alpha amylase inhibition assay.

Sl. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% Inhibition
1	Control	-	0.480	-
2	Standard (Acarbose)	200	0.306	36.25±0.01
		400	0.277	42.29±0.01
		600	0.204	57.54±0.01
3	Chloroform	200	0.351	26.87±0.01
		400	0.324	32.50±0.01
		600	0.291	39.37±0.01
4	Ethyl acetate	200	0.376	21.66±0.01
		400	0.354	26.25±0.01
		600	0.321	33.12±0.01
5	Methanol	200	0.350	27.08±0.01
		400	0.295	38.54±0.01
		600	0.229	52.29±0.01

6	Water	200	0.358	25.41±0.01
		400	0.334	30.41±0.01
		600	0.307	36.04±0.01

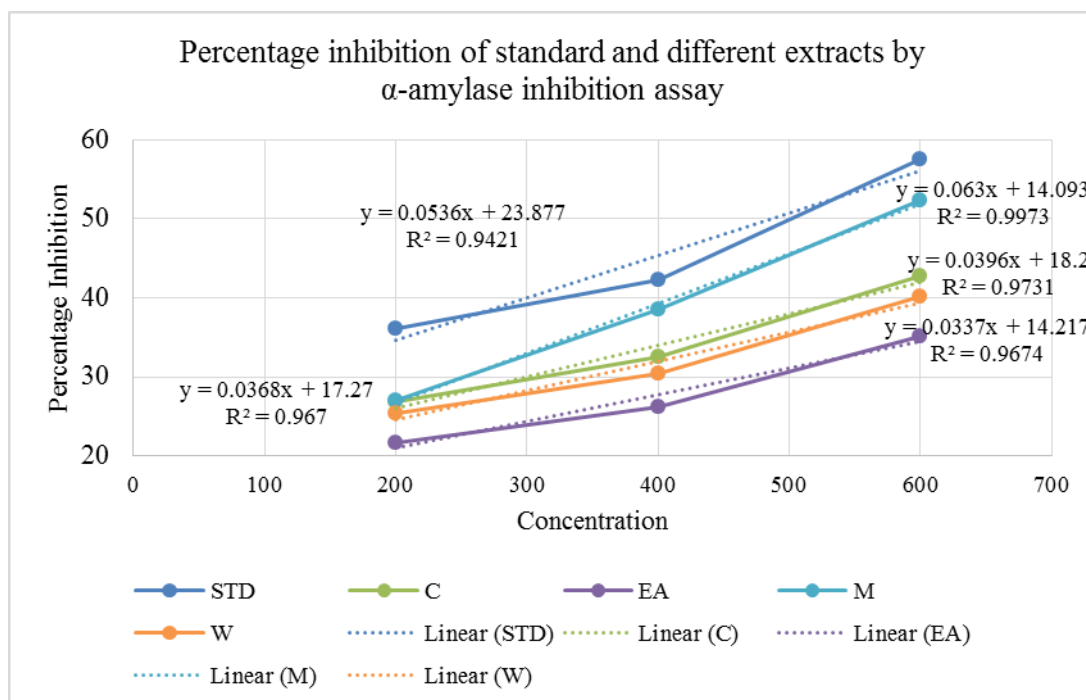


Fig No. 3: Result showing the percentage inhibition of various stem extracts of the plant *Clerodendrum chinense* by alpha amylase inhibition assay.

IC_{50} value was calculated for each extract and standard from the graph. According to Meyer et.al, the lower IC_{50} value, the more active will be the drug. Among the extracts the minimum inhibitory concentration for 50% inhibition was shown by Methanol extract. This was highly significant when compared with the concentration of the standard drug.

Table No. 9: Result showing IC_{50} values of various stem extracts of the plant *Clerodendrum chinense* by alpha amylase inhibition assay.

Sl. No.	Sample	IC_{50} (μ g/ml)
1	Acarbose (Standard)	487.3694
2	Chloroform	618.9003
3	Ethyl acetate	1061.81
4	Methanol	569.9524
5	Water	889.4022

Alpha glucosidase inhibition assay

The alpha glucosidase inhibition assay was performed by various stem extracts of the plant *Clerodendrum chinense*. The results are tabulated below.

Table No. 10: Results showing In-vitro anti-diabetic activity of various stem extracts of the plant *Cerodendrum chinense* by alpha glucosidase inhibition assay.

Sl. No	Sample	Concentration (µg/ml)	Absorbance at 400nm	% Inhibition
1	Control	-	1.989	-
2	Standard (Acarbose)	200	1.265	36.40
		400	1.142	42.58
		600	0.842	57.68
3	Chloroform	200	1.444	27.41
		400	1.308	34.21
		600	1.168	41.28
4	Ethyl acetate	200	1.532	22.98
		400	1.449	27.11
		600	1.292	35.01
5	Methanol	200	1.428	28.20
		400	1.236	37.85
		600	0.941	52.69
6	Water	200	1.461	26.54
		400	1.320	33.64
		600	1.201	39.62

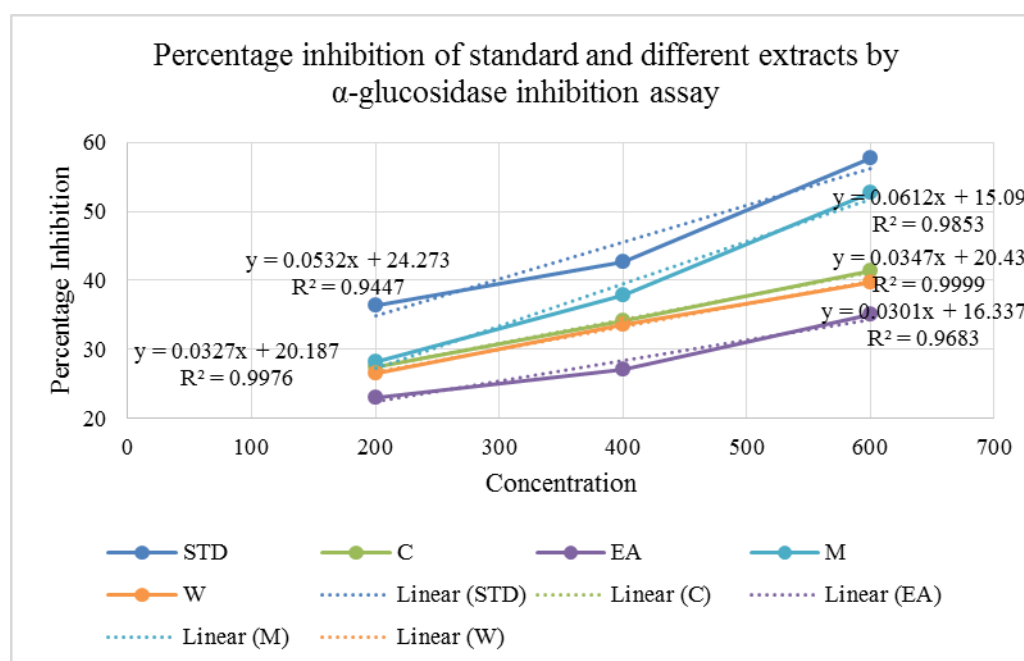


Fig No. 4: Result showing the percentage inhibition of various stem extracts of the plant *Clerodendrum chinense* by α -glucosidase Inhibition assay.

IC₅₀ value was calculated for each extract and standard from the graph. According to Meyer et.al, the lower IC₅₀ value, the more active will be the drug. Among the extracts the minimum inhibitory concentration for 50% inhibition was shown by Methanol extract. This was highly significant when compared with the concentration of the standard drug.

Table No. 11: Result showing IC₅₀ values of various extracts of stems of *Clerodendrum chinense* by alpha glucosidase inhibition assay.

Sl. No.	Sample	IC ₅₀ (µg/ml)
1	Acarbose (Standard)	483.5902
3	Chloroform	612.0622
4	Ethyl acetate	984.1359
5	Methanol	570.4248
6	Water	911.7125

SUMMARY AND CONCLUSION

Clerodendrum chinense is a member of the Lamiaceae family. A semi-woody shrub found throughout Southern Asia that goes by the names Scent malli, Brajamalli, and Chinese glory tree. It is well-known for growing wild, spreading vegetatively and being an attractive plant. The drug substance in powder form was then subjected to successive solvent extraction using solvents of increasing polarity viz. petroleum ether, chloroform, ethyl acetate, methanol and water. The dried marc was used for the next extraction after each extraction. After the solvent was distilled out of each extract, the dried extract was obtained. Preliminary phytochemical screening was done on each extract to identify the presence of phenolic compounds, flavonoids, glycosides, and saponins are present in the extracts.

Tannic acid was used as a standard in the Folin Cio-Calteau procedure to determine the total phenolic content. Methanol extracts were found to have a higher overall phenolic content.

Using quercetin as a reference, the total flavonoid concentration was determined using the Aluminium chloride colorimetric method. Methanol extract was found to have a total flavonoid content of 470 quercetin equivalents per gram. Compared to other extracts, the extracts of methanol have a higher phenol and flavonoid content.

Subsequently, all of the extracts obtained through Soxhlet extraction were employed for in vitro pharmacological investigations. Antidiabetic assay was conducted by alpha amylase inhibition assay and alpha glucosidase inhibition assay methods using four extracts viz, chloroform, ethyl acetate, methanol & water extracts. The methanolic extract possesses significant activity than other extracts when compared with the standard acarbose. Other extracts like chloroform and water extracts also shows moderate significant activity.

As a whole, it can be concluded that the stems of *Clerodendrum chinense* has a significant role in antidiabetic activity. *Clerodendrum chinense* is reported to contain phenolic compounds, flavonoids, glycosides, and saponins that may be responsible for antidiabetic activity.

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