In-vitro Anti-Diabetic and Anti-Inflammatory Activity of Leaf & Fruit Extracts of *Coccinia Indica* Linn.

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ABSTRACT

Introduction: Indian traditional system of medicine has huge varieties of various medicinal plants with pharmacological importance. "Archeologically" plants have played a significant part in medicine across the world.

Aim: The present study aims to open new avenues for the improvement of medicinal uses of the taken medicinal plant *Coccinia indica*.

Methodology: In this study, the ethanolic extracts of leaf (ELECI) and fruit (EFECI) of *Coccinia indica* have been taken to evaluate the anti-diabetic and anti-inflammatory activity.

Results: The outcome obtained shows that the extracts possessed significant level of in-vitro anti-diabetic activity by inhibition of glycosylation of haemoglobin, glucose transport across yeast cells and α -amylase inhibition. In this, inhibition of glycosylation & α -amylase was a dose dependent manner and glucose transport differs with the sample. The concentration of the glucose that remains in the medium confirms the taken plant possess significant anti-diabetic activity.

Conclusion: The study also concluded that the taken plants should possess anti-inflammatory activity through increase membrane stability of lysosome cell that has equal physiological properties with erythrocytes membrane cell and it has no hemolytic activity. However, these effects should be confirmed using in-vivo models and clinical trials for its effective utilization as therapeutic agents.

Key Words: α -amylase inhibition, Anti-diabetic activity, Anti-inflammatory activity, *Coccinia indica*, Glucose uptake, Glycosylation, Membrane stabilization

INTRODUCTION

Traditional medicine is "the knowledge skill and practices based on the theories, beliefs and experiences indigenous of different cultures, used in the maintenance of wellness and in the interference, diagnosing, betterment or treatment of material and psychological health problem."¹

Diabetes mellitus (DM) is an acute metabolic condition characterised by an increasing level of glucose in the blood result from an absolute or relative deficiency of insulin action.² DM modify a grouping of roughly 424.9 million people of age 20-79 worldwide.³ This disease is associated with micro and macrovascular complications which lead to the development of disability and life-threatening medical conditions.⁴ Hyperglycemia, hyperlipidemia, and oxidative stress are the main

important characteristics of DM and represent a major risk factor for the development of complications of diabetes.^{5,6} To date, the obtainable treatment for DM which includes insulin and other oral antidiabetic agents such as sulfonylureas, thiazolidinediones, and inhibitors of α -glucosidase. These drugs are used as monotherapy or in combination to achieve better glycemic control.⁷

The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the World. Among these 2,500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as Botanical Garden of the World⁸. It has an extraordinary diversity due to its geographic and environmental conditions. WHO established its new broad strategy of traditional medi-

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cine to help and promote less expensive, and safe of using traditional medicine and complementary alternative medicine.⁹ In Indian systems of medicine most practitioners formulate and dispense their own recipes. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments.¹⁰ Prior epidemiological studies have shown that the intake of natural antioxidants is allied with reduced risks of several diseases like diabetes and anti-inflammatory disease. Recently, there is a growing interest in finding natural antioxidants to replace synthetic ones¹¹. There are numerous herbal commodities evidenced to be having great anti-diabetic potential. *Coccinia indica* (*Bimba, cucurbitaceae & kanduri*) is renowned for its hypoglycemic and anti-diabetic properties in Ayurvedic system of medicine. *Coccinia indica*, the ivy gourd, also known as baby watermelon, little gourd, gentleman's toes, tindora or gherkin (inaccurately) is a tropical vine. It is otherwise called *Cephalandra indica*.¹² *Coccinia indica* grows abundantly all over India.

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh leaves and fruits of *Coccinia indica* was collected from agricultural field near Cheyyar and Arni, Tamilnadu. The plants were authenticated by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, West Tambaram, Chennai and the voucher specimen are stored in the library for future reference. The leaves and fruits are washed thoroughly, cut into pieces and dried in hot air oven, segregated and pulverized by mechanical grinder to fine powder and stored in a container for the following analysis.¹³

This sample 1:20 w/v was extracted using ethanol in a Soxhlet apparatus for 24 hours and concentrated using a rotary evaporator and freeze-drying in a lyophilizer till dry was obtained.^{14,15}

Preparation of stock solution of taken plant materials

10 mg of *Coccinia indica* leaves and fruits extract are taken separately and dissolved in 1ml of Dimethylsulphoxide (DMSO), which is used as stock solution with the concentration of 10,000 µg/ml, from this stock solution, different concentration of were prepared using DMSO solution.¹⁶

In-Vitro Anti-Diabetic Activity

Non-enzyme glycosylation of haemoglobin

The taken plant materials were investigated to find out the in-vitro anti-diabetic activity by estimating the degree of non-enzyme glycosylation of haemoglobin and measured calorimetrically. Glucose, haemoglobin and gentamycin solution at 2%, 0.06% & 0.02 % were prepared respectively in 0.01M

phosphate buffer at 7.4 pH. 1ml of each of above solution and 1ml of different concentrations was added to the above mixture and incubated at RT for 72 hrs. The degree of glycosylation of haemoglobin along with Alpha-Tocopherol as standard drug and measured at 520nm calorimetrically.¹⁷ All the tests were performed in triplicates. The % of inhibition was calculated by,

% of inhibition = {(Absorbance of Sample - Absorbance of Control)/Absorbance of Sample} * 100.

Glucose uptake in yeast cells

Commercial Baker's yeast purchased and prepared¹⁷, washed by repeated centrifugation (3,000xg; 5 min) with distilled water until the supernatant fluid (1-5mg) were added to 1 ml of glucose solution (5, 10 and 20mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37°C for 60 minutes, the tubes were centrifuged (2,500xg, 5 min) and glucose was estimated in the supernatant calorimetrically against the standard drug Metronidazole. All the tests were performed in triplicates. The % of increase in glucose uptake by yeast cells was calculated by,¹⁸

% in glucose increase = {(Absorbance of Sample - Absorbance of Control)/Absorbance of Sample} * 100.

α - amylase inhibition assay

The assay was based on the inhibitory activity on the starch iodine method was developed originally by.¹⁹ 1 ml of substrate - potato starch (1%w/v), 1 ml of standard drug (Acarbose) and plant extracts were taken at various concentrations, 1 ml of alpha-amylase enzyme (1%w/v) and 2 ml of acetate buffer (0.1 M, 7.2 pH) was added. The above mixture was incubated for 1 hr then 0.1 ml iodine-iodine indicator was added in the mixture and the absorbance was read at UV-Visible spectroscopy against blank. All the tests were performed in triplicates. The % of inhibition was calculated by,

% of inhibition of α - amylase = {(Absorbance of Sample - Absorbance of Control)/Absorbance of Sample} * 100.

In-Vitro Anti-Inflammatory Activity

Inhibition of albumin denaturation

The test was performed based on the methods,^{20,21} this method of protein denaturation was to assessed the anti-inflammatory activity of the taken plant extracts. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction at 37°C. The sample extracts were incubated at 37°C for 20 min and the temperature increased to 51°C for 20 minutes, cool and then the colorimetric assay was performed and the absorbance was noted at 660nm against blank. All the tests were performed in triplicates. The % of inhibition was calculated by

% of inhibition = {(Absorbance of Test - Absorbance of Control)/Absorbance of Test} * 100.

Membrane Stabilization Test

preparation of RBC suspension

The test was performed based on the methods.^{22,23} The blood (10ml) from a health volunteer who had not taken any NSAIDs for 2 weeks before experiment was collected using a vacutainer tube EDTA,²⁴ mixed with isotonic NaCl solution and centrifuged at 3000 rpm for 10 min and washed three times with the same volume of normal saline. The volume of blood was measured reconstituted as 10% suspension with normal saline.

Heat induced hemolytic assay

The test was performed based on the method, 25 ml of extract and an ml of 10% RBCs suspension were taken as reaction mixture and the aspirin is used as standard drug. The reaction mixture was consisting of test extracts were incubated in water bath for 30 min at 56°C, cooled then centrifuged at 3000rpm for 5 min and the absorbance of the supernatants was taken at 560nm. All the tests were performed in triplicates. The % of HRBC membrane stabilization activity was calculated by,

% Hemolytic = {(Absorbance of Test - Absorbance of Control)/Absorbance of Test} * 100.

RESULTS

An increase in the glycosylation was observed on incubation of hemoglobin with the increasing concentration of the glucose over a period of 72 hrs (Figure 1 & Table 1). There is significant inhibition of haemoglobin glycosylation in the taken plant extract which is indicated by the presence of increasing concentration of haemoglobin. *Coccinia indica* exhibited higher inhibition of glycosylation as compared with the taken standard drug. The plant extracts displayed the inhibition of haemoglobin glycosylation at different physiological concentration of the glucose over the period of 3 days (72 hrs), shows that the taken plant extracts minimize the formation of the glucose-haemoglobin complex and also increment the amount of free haemoglobin.

The range of glucose diffusion across the cell membrane in the yeast cells system was represented in Table & Figure 2,3 & 4 of all three-glucose concentration (20mM, 10mM and 5mM respectively). The quantity of glucose left over in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells.

The percentage inhibition at 100,80,60,40,20 µg/ml concentration of plant extract shown concentration dependent reduction in percentage inhibition. At a concentration of 20 µg/

ml ELECI & EFECI showed a percentage inhibition 52.63% and 57.14% and for 100 µg/ml ELECI & EFECI showed a percentage inhibition 87.83% and 90.00% Table 5. As the result shows ELECI & EFECI have significant activity as compared to standard drug acarbose, and 80µg/ml and 100µg/ml concentration of EFECI shows greater activity than acarbose shown in Figure 5.

As part of the investigation on the mechanism of the anti-inflammation activity, the ability of extract on protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation Table 6 & Figure 6. Maximum inhibition 89.18% was observed from EFECI and ELECI shows 82.60% at the concentration of 250 µg/ml. The taken standard drug aspirin showed the maximum inhibition 82.22% at the concentration of 250 µg/ml.

The result shows additional evidence of the anti-inflammatory effect of the taken plant extract by its membrane stabilization properties. Table 7 & Figure 7 indicated that maximum inhibition 90.42% was observed from EFECI followed by ELECI 85.93% and 81.25% of the taken standard drug Aspirin.

DISCUSSION

In-vitro non enzymatic glycosylation of hemoglobin assay of *Coccinia indica*

The leaf and fruit ethanolic extract of *Coccinia indica* shows a good anti-diabetic activity, in humans enzymatic and non-enzymatic anti-oxidative mechanisms are responsible for anti-oxidative properties that minimize the generation of ROS, which is responsible for many degenerative diseases which includes diabetics, heart diseases, cancer etc. When the concentration of glucose increases in blood it will binds to hemoglobin may results in ROS generation.²⁶ Generally, plant extracts play a major role in the inhibition of the glycosylation end products.

In-vitro glucose uptake in yeast cells

Diabetes (*madhumeha*) is one of the oldest known diseases. Since the regulation of glucose level in diabetics patients was also responsible for other complications associated with this disease. Therefore, the maintenance of glucose level is the most important regulation observed in the treatment of diabetic patients.²⁷ The *in-vitro* studies indicated that the ELECI and EFECI possess good anti-diabetic activity. The EFECI exhibited significantly higher activity than ELECI at all concentrations. However, the highest uptake of glucose was seen in 5mM glucose concentration. Results also indicated that ELECI & EFECI had greater efficiency in increasing the glucose uptake by yeast cells as compared to the taken standard drug metronidazole. It was reported that transport of non-

metabolize sugars and certain metabolizable glycosides aids the transport of glucose across the yeast cell membrane by stereospecific membrane transfer. Since the transportation of glucose in yeast is by facilitated diffusion process and transport solutes down the concentration gradients. The effective transportation is therefore attained only if there is a decrease in intercellular sugar level.

An *in-vitro* study using alpha-amylase inhibition assay

The α -amylase enzyme plays a significant role in carbohydrates digestion. There is a reduction in the postprandial glucose level on treatment of diabetic by the inhibition of α -amylase enzyme. This is one of the important strategies in the regulation of blood glucose.²⁸ The *in-vitro* α -amylase inhibition assay shows that ELECI & EFECI had good anti diabetic activity.

Inhibition of albumin denaturation by *Coccinia indica*

Inflammation is a normal protective response by your body characterized by pain, redness, heat, swelling and disturbed physiological function to an infection or injury caused by microbes, chemicals or traumas. Denatured protein generally loses its function and it will happen during inflammation.²⁹

Membrane stabilization test by *Coccinia indica*

One of the other methods to study the *in-vitro* anti-inflammatory activity using HRBC membrane stabilization which implies that the plant extract may well stabilize lysosomal membrane. Stabilization of membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated immune cells, proteases and bacterial enzymes, which will cause the tissue acute and chronic inflammation, damage and various disorders upon extracellular releases. The inhibition or stabilization of the lysosomal membrane is encountered by use of non-steroidal drugs. Stabilization of RBC membrane³⁰ is carried out using ELECI and EFECI. Both showed inhibiting properties on heat-induced hemolysis compare with the standard drug Aspirin.

CONCLUSION

It was concluded that our findings showed that both ELECI and EFECI have significant anti-diabetic activity by inhibition of glycosylation & α -amylase and glucose uptake by yeast cells and also having significant anti-inflammatory activity by membrane stabilization studies. The taken plant extracts increase the membrane stability of lysosomal cell and have similar physiological properties with erythrocyte membrane cell and it has no hemolytic activity, which con-

firms the anti-inflammatory activity of the taken plant extract. Based on this *in-vitro* studies, further *in-vivo* studies on clinical trials should be investigated.

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Table 1: *In-vitro* non-enzymatic glycosylation of haemoglobin method in ELECI and EFECI

S.No	Conc ⁿ (µg/ml)	STD			ELECI		EFECI	
		Blank Abs	Abs	% inh	Abs	% inh	Abs	% inh
1.	20		0.188±0.04*	15.42	0.194±0.02*	18.04	0.198±0.02*	19.69
2.	40		0.285±0.03*	44.21	0.313±0.03*	49.20	0.318±0.06*	50.00
3.	60	0.159±0.002	0.366±0.05*	56.55	0.412±0.06*	61.40	0.482±0.07*	67.01
4.	80		0.442±0.07*	64.02	0.498±0.05*	68.07	0.567±0.05*	71.95
5.	100		0.501±0.05*	68.26	0.567±0.04*	71.95	0.664±0.03*	76.05

STD-Standard, ELECI- Ethanolic Leaf Extract of *Coccinia indica*, EFECI-Ethanolic Fruit Extract of *Coccinia indica*, Abs-Absorbance, % inh-% inhibition, Conc. ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM

Table 2: % inhibition of Glucose uptake in 20mM glucose Concentration in ELECI and EFECI

S. No.	Concn (µg/ml)	STD			ELECI		EFECI	
		Blank Abs	Abs	% inh	Abs	% inh	Abs	% inh
1.	20	0.004±0.001	0.008±0.01*	50.00	0.007±0.04*	42.85	0.007±0.05*	42.85
2.	40		0.011±0.03*	63.33	0.014±0.03*	71.42	0.025±0.02*	84.00
3.	60		0.110±0.06*	96.36	0.124±0.05*	96.77	0.192±0.06*	97.91
4.	80		0.208±0.04*	98.07	0.204±0.04*	98.03	0.225±0.03*	98.22
5.	100		0.297±0.02*	98.65	0.298±0.03*	98.65	0.344±0.01*	98.83

STD-Standard, ELECI- Ethanolic Leaf Extract of *Coccinia indica*, EFECI-Ethanolic Fruit Extract of *Coccinia indica*, Abs-Absorbance, % inh-% inhibition, Conc. ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM

Table 3: % inhibition of Glucose uptake in 10mM glucose Concentration in ELECI and EFECI

S.No	Concn (µg/ml)	Blank	STD		ELECI		EFECI	
		Abs	Abs	% inh	Abs	% inh	Abs	% inh
1.	20	0.004±0.001	0.010±0.01*	60.00	0.014±0.02*	71.42	0.016±0.05*	75.00
2.	40		0.106±0.06*	96.22	0.187±0.01*	97.86	0.202±0.02*	98.01
3.	60		0.169±0.04*	97.63	0.220±0.07*	98.18	0.288±0.06*	98.61
4.	80		0.198±0.05*	97.97	0.285±0.03*	98.59	0.320±0.03*	98.75
5.	100		0.320±0.02*	98.75	0.404±0.04*	99.00	0.532±0.01*	99.24

STD-Standard, ELECI- Ethanolic Leaf Extract of *Coccinia indica*, EFECI-Ethanolic Fruit Extract of *Coccinia indica*, Abs-Absorbance, % inh-% inhibition, Conc.ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM

Table 4: % inhibition of Glucose uptake in 5mM glucose Concentration in ELECI and EFECI

S.No	Concn (µg/ml)	Blank	STD		ELECI		EFECI	
		Abs	Abs	% inh	Abs	% inh	Abs	% inh
1.	20	0.004±0.001	0.012±0.03*	66.66	0.015±0.04*	73.33	0.016±0.01*	75.00
2.	40		0.108±0.08*	96.29	0.270±0.05*	98.51	0.222±0.07*	98.19
3.	60		0.218±0.05*	98.16	0.395±0.09*	98.98	0.388±0.04*	98.96
4.	80		0.338±0.02*	98.81	0.498±0.05*	99.16	0.678±0.06*	99.41
5.	100		0.422±0.03*	99.05	0.663±0.04*	99.39	0.812±0.02*	99.50

STD-Standard, ELECI- Ethanolic Leaf Extract of *Coccinia indica*, EFECI-Ethanolic Fruit Extract of *Coccinia indica*, Abs-Absorbance, % inh-% inhibition, Conc.ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM

Table 5: % α-amylase inhibition in ELECI and EFECI

S.No	Concn (µg/ml)	Blank	STD		ELECI		EFECI	
		Abs	Abs	% inh	Abs	% inh	Abs	% inh
1.	20	0.009±0.002	0.017±0.02*	47.05	0.019±0.03*	52.63	0.021±0.03*	57.14
2.	40		0.021±0.06*	57.14	0.039±0.04*	76.92	0.042±0.02*	78.57
3.	60		0.042±0.05*	78.57	0.054±0.01*	83.33	0.060±0.05*	85.00
4.	80		0.057±0.04*	84.21	0.072±0.02*	87.50	0.078±0.04*	88.46
5.	100		0.068±0.06*	86.76	0.074±0.01*	87.83	0.090±0.06*	90.00

STD-Standard, ELECI- Ethanolic Leaf Extract *Coccinia indica*, EFECI-Ethanolic Fruit Extract *Coccinia indica*, Abs-Absorbance, % inh-% inhibition, Conc.ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM

Table 6: % albumin denaturation assay in ELECI and EFECI

S. No	Concn (µg/ml)	Blank	STD		ELECI		EFECI	
		Abs	Abs	% adn	Abs	% adn	Abs	% adn
1.	50	0.016±0.002	0.047±0.02*	65.95	0.068±0.03*	76.47	0.070±0.03*	77.14
2.	100		0.058±0.08*	72.41	0.074±0.08*	78.37	0.080±0.08*	80.00
3.	150		0.078±0.05*	79.48	0.084±0.01*	80.95	0.092±0.05*	82.60
4.	200		0.087±0.04*	81.60	0.088±0.02*	81.81	0.096±0.04*	83.33
5.	250		0.090±0.06*	82.22	0.092±0.01*	82.60	0.148±0.06*	89.18

STD-Standard, ELECI- Ethanolic Leaf Extract of *Coccinia indica*, EFECI-Ethanolic Fruit Extract of *Coccinia indica*, Abs-Absorbance, % adn-% albumin denaturation, Conc.ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM, IC₅₀-inhibitory concentration at 50%

Table 7: % Membrane stabilization assay in ELECI and EFECI

S.No	Concn (µg/ml)	Blank Abs	STD Abs	% ms	ELECI Abs	% ms	EFECI Abs	% ms
1.	50		0.052±0.02*	65.38	0.072±0.03*	75.00	0.078±0.03*	76.92
2.	100		0.064±0.08*	71.87	0.078±0.08*	76.92	0.088±0.08*	79.54
3.	150	0.018±0.002	0.084±0.05*	78.57	0.090±0.01*	80.00	0.096±0.05*	81.25
4.	200		0.088±0.04*	79.54	0.094±0.02*	80.85	0.144±0.04*	87.50
5.	250		0.096±0.06*	81.25	0.128±0.01*	85.93	0.188±0.06*	90.42

STD-Standard, ELECI- Ethanolic Leaf Extract *Coccinia indica*, EFECI-Ethanolic Fruit Extract *Coccinia indica*, Abs-Absorbance, % ms-% Membrane stabilization, Conc.ⁿ-Concentration. *P<0.05 when compared to control, Values are expressed as mean± SEM, IC₅₀-inhibitory concentration at 50%

Figure 1: Non-enzymatic glycosylation assay.

Figure 5: Alpha amylase inhibition assay of ELECI & EFECI

Figure 2: Glucose uptake in 20mM glucose concentration.

Figure 6: Albumin denaturation activity of ELECI & EFECI

Figure 3: Glucose uptake in 10mM glucose concentration.

Figure 7: Membrane stabilization activity of ELECI & EFECI

Figure 4: Glucose uptake in 5mM glucose concentration.