Preparation of ethanolic extract of *cassia auriculata* and its anti-diabetic activity

Running title: *cassia auriculata* and its diabetic activities

Abstract:

Introduction:
Diabetes has caused a major burden to the health sector in the developing countries and has shown an increasing trend among the urban population. It is estimated that most patients are with type II diabetes which could be easily treated with dietary changes, exercise, and medication. Sri Lanka carries a long history of ayurvedic medicine where it uses the plant for treating many diseases. Therefore it is important to screen medicinal plants scientifically so they could be used safely and effectively in the traditional medical system and also be used for further investigations. *cassia auriculata* is a plant used in the Ayurvedic medical system in Sri Lanka for treating many diseases including diabetics. We evaluated the anti-diabetic properties and the antioxidant properties of *cassia auriculata* leaves.
Methods:
The methanol extract of the leaves was sequentially extracted with petroleum ether and thereafter was partitioned between EtOAc, and water. The α-amylase inhibition assay was performed using the 3,5- dinitrosalicylic acid method. The antioxidant activities were measured using the DPPH free radical scavenging activity and the total phenolic content using Folin-Ciocalteu's reagent. The cytotoxicity of the extract was evaluated using the Brine shrimp bioassay.
Results: The extract shows very good anti-diabetic activity for the *Cassia auriculata* extract by using BSA and EAA Assay.
Conclusion:
The leaf extracts of *cassia auriculata* exhibit remarkable α-amylase inhibitory activity in the crude methanolic extract. Hence leaves of *cassia auriculata* have a potential to be used as a regular green vegetable and also be investigated further in isolating pure compounds with anti-diabetic activity.

Keywords: Cassia auriculata, Diabetic Activities, Ethanolic extract, α-Amylase Assay, α-Glucosidase Assay.

Introduction:

Diabetes is a disorder of β-cells of langerhans that can be diagnosed by its symptoms like weight loss, excessive hunger, thirst and urination(Dua, Wadhwa, Singhvi, Rapalli, Shukla, Shastri, Gupta, Satija, Mehta, Khurana, Awasthi, Maurya, Thangavelu, Rajeshkumar, *et al.*, 2019),(Ramesh *et al.*, 2018a). It is a fifth leading cause of deaths as well the leading cause of adult blindness, responsible for heart attacks, strokes and gangrenous leg amputations. *Cassia auriculata* Linn (Kingdom : Plantae, Order : Fabales, Family : Fabaceae, Sub family : Caesalpinioideae, Tribe : Cassie,Genius : Cassia,Species : C. auriculata),(Ezhilarasan, 2018a),(A. C. Gomathi *et al.*, 2020a) commonly known as Tanners Senna, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine(Rajeshkumar, Venkat Kumar, *et al.*, 2018),(Ezhilarasan, Sokal and Najimi, 2018a). The plant is used in the traditional system of medicine for urinary disorders, female antifertility, leprosy, worm infestation, diarrhoea, disease of pittam; leaves, flowers and fruits as anthelmintic; seeds for eye troubles, diabetes(Nandhini, Rajeshkumar and Mythili, 2019a).

Non-pharmacological and pharmacological methods to diabetes treatment are used(Veerasamy *et al.*, 2021). Exercise, food management, and surgery are examples of non-pharmacological approaches, whereas medicines like insulin and oral hypoglycemic treatments are examples of pharmacological approaches(Vairavel, Devaraj and Shanmugam, 2020a)s. Conventional medicines are not only expensive, but they also come with a slew of side effects(M. Gomathi *et al.*, 2020). For the treatment of diabetes, many herbal remedies have been advocated(Rajasekaran *et al.*, 2020a),(Ramesh *et al.*, 2018a). Medicinal plant components are considered to operate on a number of targets through a variety of modes and processes(*2018 Conference on Signal Processing and Communication Engineering Systems (SPACES): 4-5 Jan. 2018*, 2018),(Gheena and Ezhilarasan, 2019a).They have the potential to treat complex diseases such as diabetes and its consequences(R *et al.*, 2020),(Saravanan *et al.*, 2018a).

This research is needed to know the importance of *cassia auriculata* in anti- diabetic activity.The main deficiency it fulfill that the *Cassia auriculata* is related to histamine,kinn and prostaglandin inhibiting activity.Our team has extensive knowledge and research experience that has translate

into high quality publications(Rajeshkumar, Kumar, *et al.*, 2018; Nandhini, Rajeshkumar and Mythili, 2019b; M. Gomathi *et al.*, 2020; Rajasekaran *et al.*, 2020b; Vairavel, Devaraj and Shanmugam, 2020b),(Santhoshkumar *et al.*, 2019),(Raj R, D and S, 2020),(Saravanan *et al.*, 2018b),(Gheena and Ezhilarasan, 2019b),(Ezhilarasan, Sokal and Najimi, 2018b),(Ezhilarasan, 2018b),((Dua, Wadhwa, Singhvi, Rapalli, Shukla, Shastri, Gupta, Satija, Mehta, Khurana, Awasthi, Maurya, Thangavelu, S, *et al.*, 2019; A. C. Gomathi *et al.*, 2020b; Vairavel, Devaraj and Shanmugam, 2020b),(Ramesh *et al.*, 2018b; Duraisamy *et al.*, 2019; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Arumugam, George and Jayaseelan, 2021; Joseph and Prasanth, 2021) .,(Gnanavel, Roopan and Rajeshkumar, 2019),(Markov *et al.*, 2021)(Veerasamy *et al.*, 2021).The aim of this study is to determine anti-diabetic activity of *cassia auriculata* flower extract.

Materials and Methods:

In vitro α-amylase inhibitory studies The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method. The leaf extract of *cassia auriculata* was dissolved in minimum amount of 10% DMSO and was further dissolved in buffer ((Na2HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 10 to 1000 µg/*mL*. A volume of 200 µl of α-amylase solution (2 units/*mL*) was mixed with 200 µl of the extract and was incubated for 10 min at 30 °C. Thereafter 200 µl of the starch solution (1% in water (w/v)) was added to each tube and incubated for 3 min. The reactivity was terminated by the addition of 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 *mL* of 2 M NaOH and 20 *mL* of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 *mL* of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µl of buffer. A blank reactivity was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (100 µg/*mL*–2 µg/*mL*) and the reactivity was performed similarly to the reactivity with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph. The test was realized following the protocol of Pistia-Brueggeman and Hollingsworth. An amount of 50 µL of WFLE extract was prepared at various concentrations (1, 3, 7, 15, 31, 62, 125, 250, 500 and 1000 µg/*mL*) and incubated with the solution containing 10 µL of α-glucosidase (maltase) 1 U/*mL* and 125 µL of 0.1 M phosphate buffer (pH 6.8) for 20 min at 37 ◦C. To start the reaction, a solution of 20 µL of 1 M pNPG (4-Nitrophenylβ-D-glucopyranoside) (substrate) was added then incubated for half-hour. To terminate the reaction, 50 µL of 0.1 N Na2CO3 was added. The optical density was measured at 405 nm using a spectrophotometer. For this assay, acarbose was used as a positive control (α-amylase inhibitor).

The experiments were repeated three times for each concentration. The α- Glucosidase inhibitory activity was calculated by using the following formula: Extract inhibitory activity = [(XA − XB)/XA] × 100. XA is the absorbance of the control (100% enzyme activity) and XB is the absorbance of the sample. After determining the α-glucosidase inhibitory activity of the different concentrations, the IC50 values were determined for the acarbose and WFLE extracts (concentration required to inhibit 50% of α-glucosidase).

Results:

Image 1: *Cassia auriculata* ethanolic extract

The results of anti-diabetic activity and egg albumin assay were depicted in (Figures 1-2). In the present study, the total anti-diabetic of *Cassia auriculata ethanolic* extract (CAE) was determined using the egg albumin assay method.CAE Ext showed anti-diabetic property in a concentration dependent manner. The result indicated that the CAE Ext significantly (<0.05) inhibited albumin Denaturation Assay method.Egg albumin assay is an easy, rapid and sensitive method for the anti-diabetic screening of plant extracts. The present study investigated the anti-diabetic activity of CAE Ext, and expressed the inhibition of albumin denaturation Assay using BSA as standard reference.

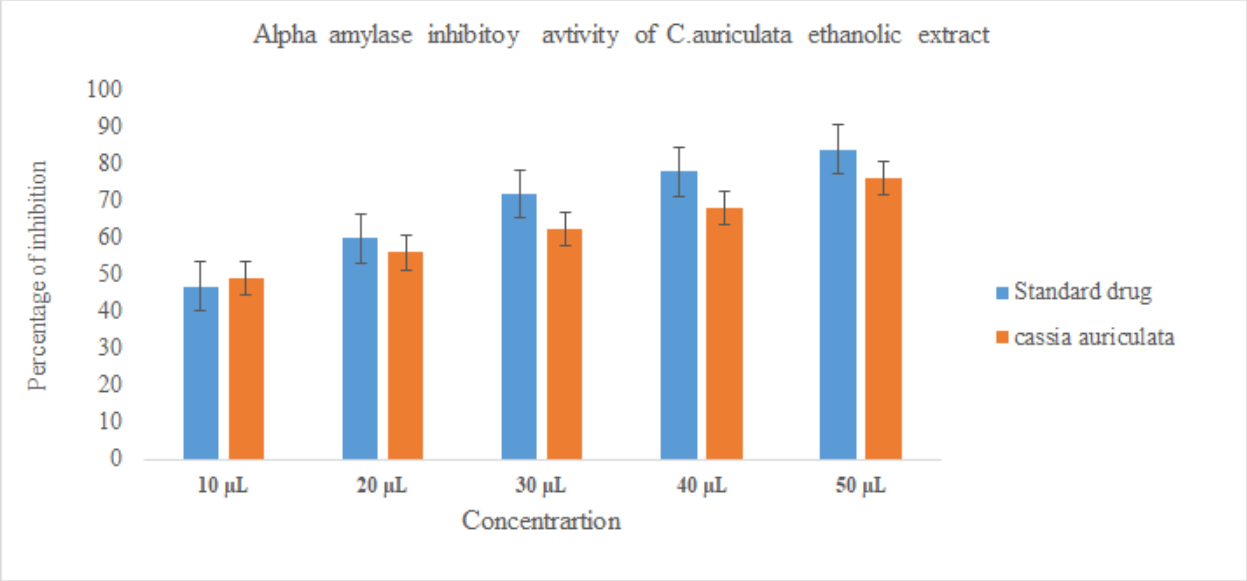

Figure 1: The above graph depicts the anti- diabetic activity with an increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Cassia auriculata*.

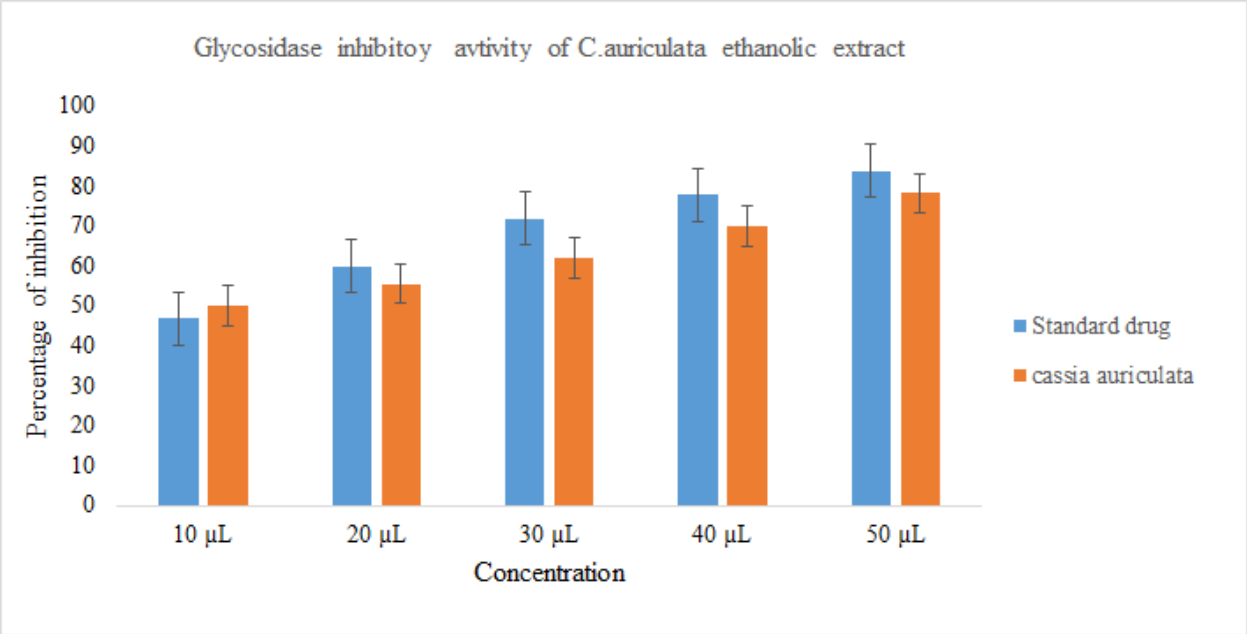

Figure 2: The above graph depicts the anti-diabetic activity of *Cassia auriculata* ethanolic extract on egg albumin assay increased percentage of inhibition with a concentration in microlitres. X axis denotes concentration and Y axis denotes percentage of inhibition of *Cassia auriculata*.

Discussion:

The *Cassia auriculata* leafs were used as a sample to evaluate the anti-diabetic activity for the reason, increased diabetic patients and there were less treatment procedures and also less drugs. They are more resistant when compared to *cassia auriculata* extracts. Hence plaque samples were used rather than standard strains.Other study done by anti-diabetic effect of Cassia auriculata, which studied the anti-diabetic property of *Cassia auriculata* has been evaluated against Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholerae and Shigella dysenteriae. But this study did not evaluate the effect of *Cassia auriculata* against diabetes. Study was done to evaluate the anti-diabetic activity of Cassia auriculata. In this study, it was shown that anti-diabetic activity.

Conclusion:

From the present study, we can conclude that ethanolic extract of *Cassia auriculata* flower extract has anti-diabetic activity . But for more precise effect in a specific way, more studies with specific strain should be done.

NOTE:

The study highlights the efficacy of " ayurvedic medicine " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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