

Antioxidant and Anti-Diabetic activities of Polyphenol-enriched Star Anise (*Illicium verum*) seeds extract

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Abstract

This study aims to determine the antioxidant and non cytotoxic activities of the spice Star Anise (*Illicium verum*) seeds. The antioxidant activities of the above evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) activity assay were used as positive control. Phytochemicals of *Star Anise (Illicium verum)* seeds, which were analyzed by using standard methods. *In vitro* anti-diabetic studies were done by alpha amylase enzyme, alpha glucosidase enzyme inhibition studies and Glucose uptake in Yeast cells studies. The phytochemical screening of *Illicium verum* revealed that the extract is rich polyphenols. The *in vitro* antidiabetic potential of extract was confirmed through α - amylase enzyme, alpha glucosidase enzyme inhibition studies and Glucose uptake in Yeast cells studies. The results of the present study concluded that *Illicium verum* shows significant antioxidant and antidiabetic activity. The above anti-diabetic activity of *Illicium verum* is due to the presence of polyphenols.

Keywords: Anti diabetic activity, Star Anise (*Illicium verum*) seeds, alpha Glucosidase Polyphenols

INTRODUCTION:

Diabetes mellitus is now life threatening throughout the world and is a metabolic disorder with increasing incidence. The inhibition activity of alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which cause a decrease in the absorption of glucose, resulting in reduction of postprandial blood

glucose level [1]. The treatment for diabetes has spent vast amount of medicines, diets, physical training and so on in all countries and also searching for new natural and synthetic compounds to overcome diabetic problems [2,3]. Star Anise (*Illicium verum*) seed is one of the popular spices which are also having medicinal properties. This seed is rich in polyphenols, proteins, flavonoids, which are responsible for its medicinal properties [4]. It is reported that, the leaves and fruits have very good antioxidant properties. The earlier studies also showed that the root extracts of the plant have antioxidant activities.

MATERIALS AND METHODS:

The required chemicals were purchased from Hi-Media Pvt. Ltd., Loba Cheme and SRL. All other chemicals used in the study were obtained commercially and were of analytical grade.

Extraction:

Star Anise (*Illicium verum*) seed were collected from authentic sources, cleaned with double distilled water, crushed, shade dried and powdered (British Pharmacopoeia 100 mesh) and stored in a glass bottle for further analysis. 10g of the above powder was mixed with 200 ml of double distilled water and vortexed for 4 hours at 20°C using magnetic stirrer. The vortexed mixture is centrifuged at 10000 rpm for 20 minutes, the supernatant was separated. The supernatant was concentrated using flash evaporator, stored at -10°C for further analysis.

Phytochemical analysis:

The aqueous extract of Star Anise (*Illicium verum*) was subjected to phytochemical analysis using following standard protocols. The proteins estimation was carried according to Bradford's method [5] using BSA as standard and absorbance was read at 535nm. Total phenols were determined according to the method of Folin Ciocalteu reaction [6] using Gallic acid as a standard and absorbance was read at 750 nm. Total Sugars estimation was done according to Dubois method [7], the absorbance was read at 520 nm. Flavonoids estimation was done using Quercetin [8] as a standard; absorbance was measured at 415 nm. The concentrations were calculated accordingly using standard graph.

Antioxidant activity:

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was assessed according to the method of Shimada et al. with minor modifications [9] at a concentration of 25µg each was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer

pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured spectrophotometrically at 517 nm. BHA (400 µM), Ascorbic acid (400 µM) and α -tocopherol (400 µM) was used as positive control under the same assay conditions. Negative control was without any inhibitor or *Illicium verum* extract. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of extracts of *Illicium verum* was calculated from the decrease in absorbance at 517 nm in comparison with negative control.

***In vitro* methods employed in anti-diabetic 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 this, 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 [10-12].

Inhibition of alpha glucosidase enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of *Illicium verum* extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α -glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method [13-15]

Glucose uptake in Yeast cells

Yeast cells were prepared according to the method of Gupta et al., 2013 with minor modifications [16]. Yeast cells briefly, commercial baker's yeast was washed in distilled water by repeated centrifugation till the supernatant clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of *Illicium verum* (1–5 mg) were added to 1mL of glucose solution (5, 10 and 25 mM) 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 min. After 60 min, the tubes were centrifuged and glucose was estimated in the supernatant. Metformin was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated. All the tests were performed in triplicate [17].

Statistical analysis

Statistical analysis was done in SPSS (Windows Version 10.0.1 Software Inc., New York) using a one-sided student's t-test. All results refer to means \pm SD. $P < 0.05$ was considered as statistically significant when compared to relevant controls.

RESULTS AND DISCUSSION:

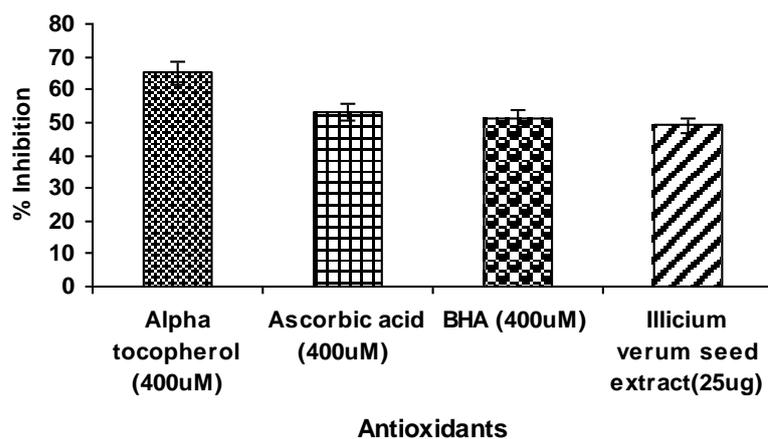


Figure 1: DPPH radical scavenging activity of *Illicium verum* seed extract.

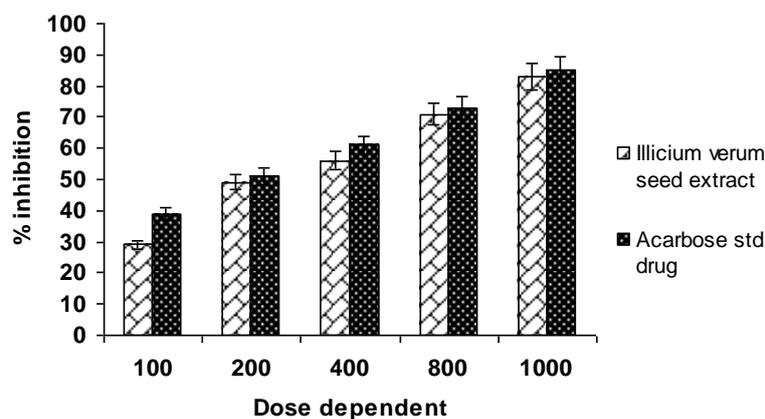


Figure.-2: α -amylase inhibitory activity by *Illicium verum* seed extract

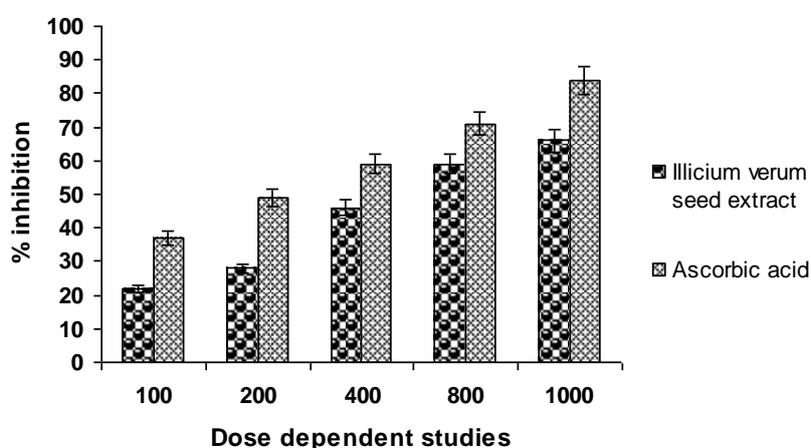


Figure 3: The *in vitro* alpha-glucosidase inhibitory activity of *Illicium verum* seed extract

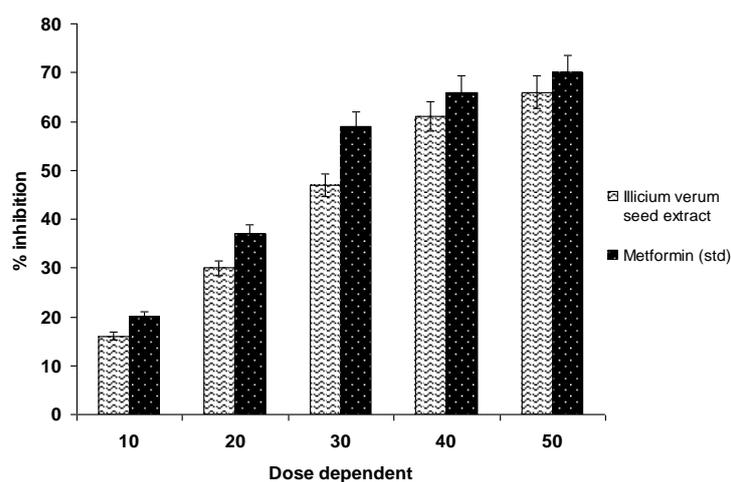


Figure -4: Glucose uptake in yeast cells by *Illicium verum* seed extract

Illicium verum seed extract was done using double distilled water extract of as explained in methods. The analysis result showed that, the extract rich in polyphenols. The *Illicium verum* seed extract was analyzed for its antioxidant activity by DPPH radical scavenging activity where α -tocopherol, Ascorbic acid and BHA were used as standard antioxidants at a maximum dosage of 400 μ M and *Illicium verum* seed extract used at a dosage of 25 μ g. **Figure -1** showed that, α -tocopherol, Ascorbic acid, BHA and the *Illicium verum* seed extract showed inhibition of DPPH radicals. The inhibition of the *Illicium verum* seed extract is comparable with standard antioxidants. This means, the crude *Illicium verum* seed extract have good antioxidant activity when compared to standards.

The dose-dependent *invitro* α -amylase inhibitory activity of *Illicium verum* seed extract was done as explained in methods. It was found that, there is increase in

percentage inhibitory activity with the increase in dosage against α -amylase enzyme. As a standard drug, Acarbose was used with similar dosage to compare inhibitory capacity of *Illicium verum* seed extract. **Figure -2** showed that, the % inhibitory activity of *Illicium verum* seed extract ranges a minimum of 28.11 ± 0.12 (at $100 \mu\text{g/ml}$) to a maximum of 85.23 ± 0.23 (at $1000 \mu\text{g/ml}$) where as the standard drug Acarbose showed % inhibitory activity ranges from 26.14 ± 0.11 (at $100 \mu\text{g/ml}$) to a maximum of 64.12 (at $1000 \mu\text{g/ml}$). The IC₅₀ value of *Illicium verum* seed extract was 532.63 ± 1.4 and the standard drug Acarbose was found to be $299.22 \pm 1.3 \mu\text{g/ml}$.

The *invitro* α -glucosidase inhibitory activity of *Illicium verum* seed extract was studied as explained in methods. It was found that, there is increase in percentage inhibitory activity with the increase in dosage against α -glucosidase. As a standard drug, Acarbose was used with similar dosage to compare inhibitory capacity of the *Illicium verum* seed extract. **Figure -3** showed that, the % inhibitory activity of *Illicium verum* seed extract ranges a minimum of 21.13 ± 0.22 (at $100 \mu\text{g/ml}$) to a maximum of 69.31 ± 0.21 (at $1000 \mu\text{g/ml}$) where as the standard drug Acarbose showed % inhibitory activity ranges from 35.11 ± 0.13 (at $100 \mu\text{g/ml}$) to a maximum of 82.21 (at $1000 \mu\text{g/ml}$). The IC₅₀ value of *Illicium verum* seed extract was 392.13 ± 1.3 and the standard drug acarbose was found to be $266.21 \pm 1.2 \mu\text{g/ml}$.

The rate of glucose transport across cell membrane in yeast cells system is as presented in **Figure-4**. In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place via diffusion. Diabetes like type -II is described as the deficiency of insulin resulting in increased amount of glucose in blood. After the treatment of the yeast cells with these *Illicium verum* seed extract in a dose dependent manner, the glucose uptake was found to increase. The % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively. The *Illicium verum* seed extract exhibited significant activity at all glucose concentrations in comparison with Standard drug Metformin.

CONCLUSION:

The results of the present study demonstrated that the phytochemical analysis of *Illicium verum* seed extract are rich with polyphenols. The *invitro* antidiabetic potential of *Illicium verum* seed extract was confirmed through different model systems and to compare the activity standard drugs are used. Hence, further purification to find active polyphenol which is responsible for antidiabetic activity has to be done along with in vivo studies.

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