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Studies on Anti-diabetic Activities in Flowers of Avaram, *Senna auriculata* (L.) Roxb.

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Abstract

The research project on "Studies on anti-diabetic activities in flowers, leaves and barks of Avaram, *Senna auriculata* (L.) Roxb." was carried out at Horticultural College and Research Institute for Women, Tamil Nadu Agricultural University, Tiruchirappalli during 2015–16. Avaram is used in the treatment of diabetes in Siddha and Ayurvedic medicines. The flowers, leaves and bark of Avaram were collected from Medicinal Park, Horticultural College and Research Institute for Women, Tiruchirappalli and Krishnagiri. The methanol extract of flowers exhibited high alpha-amylase inhibition compared to leaves and barks. The highest α-amylase inhibition was recorded in flowers at 40 µg ml^{-1} (86.2%) followed by 30 µg ml^{-1} (85.5%) with IC_{50} value of 21 µg ml^{-1}. The highest inhibition was recorded in leaves of Avaram at 40 µg ml^{-1} (71.11%) followed by 30 µg ml^{-1} (69.25%) with IC_{50} value of 19 µg ml^{-1}. The highest inhibition was recorded in the bark of Avaram at 40 µg ml^{-1} (64%) followed by 30 µg ml^{-1} (62.62%) with IC_{50} value of 22 µg ml^{-1}. DPPH radical scavenging activity was found to be high in flowers compared to leaves and barks. The highest radical scavenging activity was recorded in flowers at 80 µg ml^{-1} (73.78%) sample followed by 100 µg ml^{-1} (73.32 %) with IC_{50} value of 55 µg ml^{-1}. In GC-MS/MS analysis of flowers, the highest peak area was recorded for Myo-inositol 4-C- methyl (53.91 %) followed by resorcinol (20.71%). The anti-diabetic compounds include Myo-inositol 4-C methyl, resorcinol, polyphenols and sterols.

Keywords: α-Amylase inhibition, anti-diabetic compounds, antioxidants, myo-inositol, *Senna auriculata*

1. Introduction

Diabetes mellitus is a syndrome and serious metabolic disorder which is caused by defects in either insulin secretion, insulin action, or both and results in hyperglycemia. Diabetes mellitus means "Siphoning off sweet water". The types of diabetes include type 1, type 2 and gestational diabetes. Type 2 diabetes is the most common type of diabetes in adults. Among three types, type 2 diabetes accounts for 90% of diabetes in adults and caused by insufficient insulin secretion by the pancreas and insulin resistance. Uncontrolled diabetes can cause many chronic complications mainly blindness, heart disease and renal failure leading to death. In the year 1990, 26 million Indian people got affected by diabetes and the number is increasing at a faster rate. As of 2019, 77 million people got affected by diabetes in India. It is predicted that diabetes may badly affect 134 million Indians by 2045. Diabetic patients mainly depend on allopathic medicines for their survival. More than 70% of diabetic patients use allopathic medicines which often cause side effects. Medicines and counter medicines to treat side effects are extremely dangerous to the human body. The increasing trend of diabetes will continue to exist in low and middle-income groups. These sections of people are not able to afford for buying costly allopathic medicines. In this situation, the search for cost-effective alternative medicines with 'No side effects' is essential to save millions of people. Plants have been used in traditional medicine for treating diabetes (Rhemann and Zaman, 1989; Pari and Latha, 2002) by stimulating the pancreas to produce insulin. Avaram, *Sennna auriculata* (L.) Roxb.has medicinal values. (Joy et al., 2012). It is used for curing diabetes (Mhod et al., 2017) and regulating cholesterol metabolism (Vijayakumar and Nachiappan, 2017). Flowers and buds are used for preparing tea instead of normal tea for diabetic patients. Seeds of this species also possess the anti-diabetic activity (Nanumala et al., 2015). Avaram belongs to the family Fabaceae. It is also known as Tanner's Cassia. It is an herbaceous plant that is found throughout central and southern India. The shrub usually occurs on roadsides, wastelands and railway embankments. The flower has been reported to contain flavonoids, proanthocyanidins and β-sitosterol. Flavonoids (Lodhi and Kori, 2021), sterols (Ramith et al., 2016), terpenoids (Panigrahy et al., 2020) and phenolic acids are known to be bioactive anti-diabetic principles. Flavonoids are known to regenerate the damaged beta cells. Phenolic compounds are found to be effective

antihyperglycemic agents. The alpha-amylase and glycosidase inhibitory potential offer a prospective therapeutic approach for the management of diabetes (Shravan Kumar et al., 2015). The leaves and flowers of *Cassia auriculata* (L.) Roxb. possess significant anti-diabetic activity (Gayathri et al., 2018) along with potent antioxidant potential in diabetic conditions (Anushia et al., 2009; Doshi et al., 2011; Kulkarni et al., 2015) and hepatoprotective property (Guruprasad et al., 2015). β-Sitosterol is used to treat diabetes (Babu and Jayaraman, 2020).

2. Materials and Methods

The research work on "Phytochemical analysis of Avaram, *Senna auriculata* (L) Roxb. for anti-diabetic compounds" was carried out at Horticultural College and Research Institute for Women, Tamil Nadu Agricultural University, Tiruchirappalli during 2015–16. The flowers, leaves and bark of Avaram were collected from Medicinal Park, Horticultural College and Research Institute for Women, Tiruchirappalli and Krishnagiri. The flowers, leaves and bark were dried under shade for 4-5 days and ground into powder. The dried powder of flowers, leaves and bark was taken for further analysis.

The powder of flowers, leaves and bark of Avaram collected from Horticultural College and Research Institute for Women, Tiruchirappalli and Krishnagiri were taken for sample preparation. Methanol was added to the beaker containing powder of flowers, leaves and barks separately and closed with aluminum foil. The powder was soaked in methanol overnight with frequent stirring. The transparent solution was collected and filtered completely through filter paper, Whatman No.1. The collected filtrate was poured into Petri dish and allowed to evaporate completely. After evaporation of methanol, the fine powder was taken for α-amylase inhibition and DPPH radical scavenging activity.

2.1.1. Alpha-amylase inhibitor assay

Alpha-amylase inhibitor assay was carried out using a modified procedure of McCue and Shetty, 2004. The series of 10, 20, 30 and 40 µg of powder (Flower, leaves and bark separately) was taken in a beaker. A volume of 500 µl of 0.02 M sodium phosphate buffer containing α-amylase solution (1 mg in 10 ml of sodium phosphate buffer) was added and pre-incubated at 25°C for 10 minutes. 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer was added, further incubated at 25°C for 10 minutes. After incubation, 500 µL of dinitrosalicylic acid (DNS) reagent was added to terminate reaction. Then it was incubated in the water bath for 5 minutes and cooled at room temperature. Then the resultant solution was diluted with 5 ml of distilled water. Then, the absorbance was measured at 540 nm using a UV spectrophotometer. A control sample was prepared by replacing the extract with distilled water. The inhibitory activity of α-amylase was calculated in percentage.

Amylase inhibition (%)= $(A_c - A_e)/A_c \times 100$

A_c - Absorbance of the control, A_e - Absorbance of the extract

Concentrations of extracts resulting in 50% inhibition of enzyme activity were determined graphically.

2.1.2. DPPH radical scavenging activity

Different concentration of the extracts 20, 40, 60, 80 and 100 µg/ml was mixed with 1 ml of DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (0.2 Mm ml^{-1} in methanol). The solution was incubated for 20-40 minutes in dark condition. Using a UV spectrophotometer, the absorbance of solution was measured at 517 nm.

DPPH Radical scavenging (%) = $(A_c - A_t) /A_t \times 100$

A_c - Absorbance of the control

A_t - Absorbance of the test sample

IC_{50} value was defined as the concentration of extracts that inhibits the formation of DPPH radicals by 50%. IC_{50} was calculated for all the extracts by plotting the percentage of DPPH radicals versus concentration of extract. Low IC_{50} value represents high radical scavenging effect.

2.2. Identification of phytochemicals using GC-MS/MS

The identification of phytochemicals was done using GC-MS/MS. About 25 g of flower powder soaked in 30 ml of ethanol overnight then filtered through the filter. The filtrate was concentrated by flushing nitrogen gas and concentrated to 1 ml. The concentrate was again filtered in the Whatman No.41 filter paper along with 2 g Sodium sulfate to remove the sediments and traces of water in the filtrate. The ethanolic extract of flower powder was analyzed through Gas Chromatography-Mass Spectrometry/Mass Spectrometry GC-MS/MS) for chemical composition. The GC-MS is a Scion 436- GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/95% Dimethylpolysiloxane) and Length: 30m; Internal diameter: 0.25 mm; Thickness: 0.25 µm. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (split ratio of 10:1). The column oven temperature program was as follows: 110°C hold for 3.50 min, Up to 200°C at the rate of 10°C/min-No hold, Up to 280°C at the rate of 5°C/min - 9 min hold, Injector temperature 280°C and total GC running time was 37.50 minutes. The solvent delay was 0-3.5 min. A scan interval of 0.5 seconds and mass scan from m/z 50 to 500 amu was programmed. The inlet line temperature was set at 290°C, source temperature 250°C. The relative amount of components was calculated by comparing their average peak area to the total areas.

3. Results and Discussion

Antioxidant and carbohydrate hydrolyzing enzyme inhibitor properties are responsible for anti-diabetic activity. The results of α-amylase inhibitor activity, DPPH scavenging activity and identification of phytochemicals using GC - MS/MS are given. The α-amylase inhibitor activity of methanolic extract of flowers, leaves and barks of Avaram collected from

Tiruchirappalli was depicted in Table 1. The increase in the concentration of flower, leaf and bark extract showed the increase in the inhibition of α-amylase. The highest inhibition was recorded in flowers of Avaram at 40 µg ml^{-1} (86.2%) followed by 30 µg ml^{-1} (85.5%) with IC_{50} value of 21 µg ml^{-1} (Table 3). The highest inhibition was recorded in leaves of Avaram at 40 µg ml^{-1} (71%) followed by 30 µg ml^{-1} (69 %) with IC_{50} value of 20 µg ml^{-1}. The highest inhibition was recorded in the bark of Avaram at 40 µg ml^{-1} (70.51%) followed by 30 µg ml^{-1} (70.36%) with IC_{50} value of 26 µg ml^{-1}. The α-amylase inhibitor activity of methanolic extract of flowers, leaves and barks of Avaram collected from Krishnagiri was depicted in Table 1. The increase in the concentration of flower, leaf and bark extract showed the increase in the inhibition of α-amylase. The highest inhibition was recorded in flowers of Tanners cassia at 30 µg ml^{-1} (91.41%) followed by 40 µg ml^{-1} (91.03%) with IC_{50} value of 18 µg ml^{-1} (Table 3). The highest inhibition was recorded in leaves of Avaram at 40 µg ml^{-1} (71.11 %) followed by 30 µg ml^{-1} (69.25 %) with IC_{50} value of 19 µg ml^{-1}. The highest inhibition was recorded in the bark of Avaram at 40 µg ml^{-1} (64 %) followed by 30 µg ml^{-1} (62.62%) with IC_{50} value of 22 µg `ml^{-1}. The possible mechanism of ethanol extract for antihyperglycemic action may be the induction of pancreatic insulin secretion from beta cells of islets of Langerhans and enhanced transport of blood glucose to peripheral tissue. The flower extract of Avaram is effective in the treatment of diabetes due to the high carbohydrate hydrolyzing enzyme inhibitor activity (Jyothi et al., 2012) which is responsible for the slow conversion of starch into glucose in our body (Kumar et al., 2011). The alpha-amylase inhibitor activity of methanolic extract of flowers of Avaram collected from Krishnagiri was highest when compared to flowers Avaram collected from Tiruchirappalli.

3.1. DPPH radical scavenging activity

Antioxidants are molecules which is capable of trapping free radicals. DPPH radical scavenging activity measures the antioxidant activity of the extract.

The DPPH radical scavenging activity of flowers, leaves and barks of Avaram, *Senna auriculata* collected from

Table 1: Estimation of α-amylase inhibition (%) in flowers, leaves and barks of Avaram, *Senna auriculata* (L.) Roxb. collected from Tiruchirappalli and Krishnagiri

Sl. No.	Concentration of extracts (µg ml^{-1})	Inhibition (%) in plant parts collected from Tiruchirappalli			Inhibition (%) in plant parts collected from Krishnagiri		
		Flowers	Leaves	Barks	Flowers	Leaves	Barks
1.	10	82.00	63.00	53.25	85.74	63.82	56.27
2.	20	83.00	67.00	57.56	89.89	67.48	57.64
3.	30	85.50	69.00	70.36	91.41	69.25	62.62
4.	40	86.20	71.00	70.51	91.03	71.11	64.00

Table 2: IC50 value (µg ml^{-1}) for α-amylase inhibition in flowers, leaves and barks of Avaram, *Senna auriculata* (L.) Roxb. collected from Tiruchirappalli and Krishnagiri

Sl. No.	Plant extracts	IC50 value (µg ml^{-1})	
		Plant parts collected from Tiruchirappalli	Plant parts collected from Krishnagiri
1.	Flowers	21	18
2.	Leaves	18	19
3.	Barks	19	22

Table 3: DPPH scavenging activity (%) in the flowers, leaves and barks of Avaram, *Senna auriculata* (L.) Roxb. collected from Tiruchirappalli

Sl. No.	Concentration of extracts (µg ml^{-1})	DPPH scavenging activity (%)		
		Flowers	Leaves	Barks
1.	20	59.29	46.64	48.00
2.	40	59.60	43.14	46.30
3.	60	67.53	39.02	33.00
4.	80	73.78	35.97	32.77
5.	100	73.32	31.40	24.29

Tiruchirappalli was depicted in Table 3. The radical scavenging activity is increasing with the concentration of the flower extract. The highest radical scavenging activity was recorded in flowers of Avaram at 80 µg ml^{-1} (73.78%) sample followed by 100 µg ml^{-1} (73.32%) with IC_{50} value of 55 µg ml^{-1}. Phenols are responsible for anti-oxidant activity (Purushotham et al., 2014). The highest radical scavenging activity is due to the presence of flavonoids and phenols (Sekhar and Anju, 2014).

The radical scavenging activity is decreasing with the concentration of the leaf and bark extract. The highest radical scavenging activity was recorded in leaves of Avaram at 20 µg ml^{-1} (46.64%) sample followed by 40 µg ml^{-1} (43.14%) with IC_{50} value of 60 µg/ml. The highest radical scavenging activity was recorded in the bark of Avaram at 20 µg ml^{-1} (48%) sample followed by 40 µg ml^{-1} (46.3%) with IC_{50} value of 68 µg ml^{-1}. The decreasing trend of radical scavenging activity in the leaves and barks may be due to the inhibitory effect of other chemicals present.

From the results, it is clear that the DPPH radical scavenging activity was highest in the flowers compared to the leaves and barks of Avaram. The highest radical scavenging activity in the flowers (Joshi et al., 2015) may be due to the presence of more antioxidants (Heim et al., 2002), total phenolics and flavonoids. Among flower, leaf and bark extracts, methanolic flower extract has a least IC_{50} value which represents the highest DPPH radical scavenging activity compared to leaves and barks.

Avaram flowers are useful in scavenging the free radicals and toxic intermediates of incomplete oxidation in the body (Jeyashanthi and Ashok, 2010). Free radicals are produced more in diabetic patients (Jain et al., 1998). The antioxidants protect the body from the adverse effects of free radicals produced (Halliwell and Gutteridge, 1990).

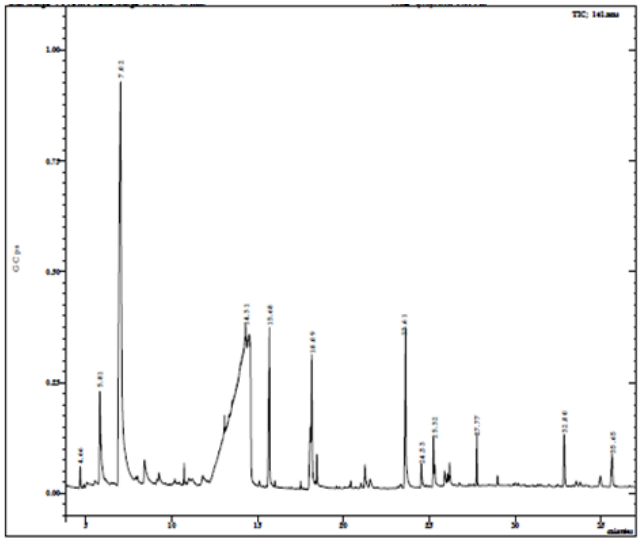

Figure 1: GC-MS/MS chromatogram of Avaram flowers collected from Tiruchirappalli

3.2. Identification of phytochemicals in avaram flowers using GC-MS/MS

The flower powder was analyzed using Gas Chromatography-Mass Spectrometry (Scion 436-GC Bruker model coupled with a column of BR-5MS, TQ quadruple mass spectrophotometer) and NIST version 11 library. Twenty six compounds were identified from flower powder. Figure 1 showed the presence of phytochemicals in the flowers of Avaram. The major phytochemicals are Myo-inositol, 4-C-methyl and Resorcinol. The highest peak was observed for Myo-inositol, 4-C-methyl (53.91%) with a molecular weight of 194 g mol^{-1}. followed by Resorcinol (20.71%) with a molecular weight of 110 g mol^{-1}.

The major group of phytochemicals belongs to sugar alcohol, polyphenols, unsaturated fatty acids, saturated fatty acids and sterols. The majority of the anti-diabetic compounds belong to Myo-inositol 4-C methyl, polyphenols, sterols, antioxidants and amylase inhibitors. Several authors reported that flavonoids, terpenoids, phenolic acids, sterols are known to have bioactive anti-diabetic properties (Rhemann and Zaman, 1989). Phenolic compounds are effective in the treatment of diabetes (Sekhar and Anju, 2014). Polyphenols are involved in the regeneration of damaged beta cells. Myo-inositol 4-C methyl reduces insulin resistance. It is involved in insulin signal transduction. Amylase inhibitors slow down the conversion of starch and glycogen to glucose. Sterols lower low-density fats which will reduce the complication of diabetes. The anti-diabetic effect of ethanol extract of flowers may be due to the presence of more anti-diabetic compounds and their synergistic effects.

4. Conclusion

Avaram is being used in the treatment of diabetes. The methanol extract of flowers exhibited high α-amylase inhibitor potential compared to leaves and bark. DPPH radical scavenging activity was found to be high in flowers compared to leaves and bark. From GC-MS/MS analysis, the highest peak area was recorded for Myo-inositol 4–C- methyl (53.91%). This study will be useful for pharmaceutical industries. The anti-diabetic compounds may be isolated and used as safe, efficacious, acceptable medicine for diabetic patients.

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