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In vitro Anti-Diabetic and Antioxidant Potential of the Siddha formulation *Mega Sanjeevi Chooranam*

Research Article

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Abstract

Background: The increasing prevalence of type 2 diabetes mellitus (T2DM) presents a significant public health challenge due to its long-term complications and its role as a primary risk factor for cardiovascular diseases, which have both economic and social implications. Plant-derived antidiabetic drugs are being extensively studied due to their safety and efficacy. The main objective of the present study is to evaluate the anti-diabetic and anti-oxidant potential of the formulation *Mega Sanjeevi Chooranam* (MSC) through *in-vitro* investigation. Materials and Methods: The *in vitro* investigation of traditional siddha formulation MSC were performed to ascertain its inhibitory potential against α-amylase and α-glucosidase enzymes along with antioxidant analysis. Results: The MSC displayed the highest activity against α-amylase enzyme, with a percentage inhibition of 73.44 ± 6.87% (IC_{50}, 299.4 ± 27.27 µg/ml), followed by a moderate level of α-glucosidase inhibition at 48.89 ± 13.38% (IC_{50}, 402.2 ± 86 µg/ml). Furthermore, the MSC exhibited the strongest antioxidant activity in quenching ABTS• radicals (IC_{50}, 110.1 ± 18.6 µg/ml), followed by DPPH• (IC_{50}, 144.4 ± 36.8 µg/ml), H_2O_2• (IC_{50}, 172.7 ± 39.3 µg/ml), and NO• radicals (IC_{50}, 238.4 ± 58.56 µg/ml). Conclusion: In conclusion the formulation MSC demonstrated the significant antioxidant activity and effectively inhibited α-amylase and α-glucosidase enzyme activities. Further study is needed to isolate bioactive phytoconstituents and determine the molecular mechanism behind their antidiabetic effect.

Keywords: Type 2 diabetes, *Mega Sanjeevi Chooranam*, Anti-diabetic, Antioxidant activity, Enzyme Inhibition, Alpha-glucosidase, Alpha-amylase, Siddha.

Introduction

Diabetes and its complications impose a substantial economic burden on individuals, families, the healthcare system, and the national economy. In 2019, the global direct healthcare costs associated with diabetes reached a staggering US$760 billion. Experts predict that these costs will continue to climb, reaching an estimated US$825 billion by 2030 and US$845 billion by 2045. (1) T2DM poses a significant challenge to public health, given its rising prevalence, long-term complications, and its association with cardiovascular diseases, which have wide-ranging economic and social consequences. While synthetic drugs have proven to be effective in managing diabetes, it is important to note that they can also come with a range of side effects. Pharmacological substances derived from plants have shown significant potential in the treatment and prevention of type 2 diabetes. (2)

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Previous research has indicated that the use of synthetic antidiabetic drugs to treat type 2 diabetes can lead to negative effects. As an example, heart failure can be caused by thiazolidinedione, while hypoglycemia and weight gain are potential side effects of sulfonylureas. (3) The fascination with natural biologically active compounds stems from the understanding that they possess minimal side effects in comparison to synthetic compounds. Moreover, research conducted in recent decades has highlighted the significance of the synergistic impact of bioactive compounds found in natural mixtures. (4) Given the exorbitant prices and potential negative effects associated with synthetic antidiabetic medications, it is imperative to explore more environmentally friendly and cost-effective alternatives for effectively managing this condition.

Herbal medicines play a vital role in global healthcare, serving as both a traditional and complementary form of therapy, as well as a valuable source of innovative pharmaceuticals. However, the global investment in research on herbal medicine is not commensurate with the significant reliance on plant-based therapies worldwide. (5)

Given the significant role that oxidative processes play in the development of metabolic diseases and age-related degenerative disorders, there is a growing interest in exploring the potential of herbs and spices as

sources of antioxidants for the treatment of various ailments, including diabetes. (6)

Plant-derived antioxidants have been found to effectively protect β cells by inhibiting the peroxidation chain reaction. This quality attributes to limit the oxidative stress in people with diabetes. (7) Considering the limitations of the currently available drugs used in the treatment of diabetes, it is worth noting that herbal medicines have been widely used and recommended for this purpose. These natural remedies contain different phytoconstituents that act on various targets through different mechanisms, resulting in therapeutic effects. (8)

Numerous reports have been made on the different manners in which certain plants and dietary compounds can help with diabetes. These include promoting insulin secretion, improving insulin sensitivity, activating protein kinase activity, regulating glucose and lipid metabolism, and inhibiting certain enzymes like α-amylase and α-glucosidase. (9,10) India stands out for its possession of six distinct medical systems that are widely recognised in this field. Some of the practices include Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homoeopathy. Although the siddha system of medicine is highly valued and deeply ingrained in Indian culture, it has also gained recognition worldwide for its effective approach in treating serious illnesses. (11)

The Siddha system of medicine has been a trusted healthcare practice for centuries, effectively meeting the needs of people throughout the ages. The foundation of siddha treatment centres around the identification of the underlying factors that contribute to the development of diseases, taking into account the condition of *Vata*, *Pitha*, and *Kaba*. According to the principles of traditional medicine, any changes in the *tridosha* can lead to metabolic changes in the body, potentially resulting in various diseases. (12) Versatile siddha formulations listed in literature have gained attention in recent times one such potential formulation is *Mega Sanjeevi Chooranam* (MSC) a polyherbal preparation comprises of ten herbal ingredients namely Strychnos Potatorum, Eugenia jambolana, Plectranthus vettiveroides, Vetiveria zizanioides, Syzigium aromaticum, Anacyclus pyrethrum, Cassia fistula, Cassia auriculata, Gymnema sylvestre and Tinospora cordifolia. The main objective of the present study is to evaluate the anti-diabetic and anti-oxidant potential of the formulation MSC through *in-vitro* investigation.

Materials and methods

Chemicals and Reagents

1,1-diphenyl-2-picryl hydrazyl (DPPH), Methanol, Potassium persulphate, phosphate buffer, Hydrogen peroxide, Butylated hydroxyanisole (BHA), Ascorbic acid, Gallic acid, Phosphoric acid, Naphthylethylene diamine dihydrochloride, Sodium nitroprusside, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Dimethyl sulfoxide, p-nitro-phenyl-α-D-glucopyranoside (p-NPG), Sodium carbonate (Na_2CO_3), Sodium chloride, di-sodium hydrogen phosphate was purchased from Hi-Media, Mumbai. Enzymes including α-glucosidase enzyme derived from Saccharomyces cerevisiae, α-amylase enzyme obtained from procaine pancreas, and 3, 5-di-nitro salicylic acid (DNSA) were procured from Sigma-Aldrich, Bangalore. The polyherbal siddha formulation known as *Mega Sanjeevi Chooranam* was prepared in the Gunapadam Lab of National Institute of Siddha, Chennai, Tamil Nadu, India. All the ingredients of *Mega Sanjeevi Chooranam* (MSC) are purified individually, powdered separately into fine powder and mixed in prescribed proportion. Then it is stored in an air tight container and labelled as *Mega Sanjeevi Chooranam* (MSC).

Determination of antioxidant Potential by DPPH assay

The antioxidant ability of the MSC formulation was assessed utilising *in vitro* 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity. (13, 14) In this investigation, 0.004 g of DPPH crystalline solid was dissolved in 100 mL of analytical grade methanol to produce a 0.1 mM DPPH solution. The resultant solution was then kept at 4 degrees Celsius. To make concentrated solutions (10-100 µg/mL), the sample MSC was dissolved in methanol. Approximately 2 mL of sample solution (MSC) from each concentration was aliquoted into separate test tubes, followed by 3 mL of DPPH solution in each. Following a 30-minute incubation period in a light-restricted environment, the absorbance at 517 nm was measured using a UV-Visible Spectrophotometer. This study used ascorbic acid as the reference standard, with values ranging from 50 to 300 µg/mL. A control solution was made by mixing 3 mL of 0.1 mM DPPH with 100 µL of MeOH. The trials were repeated three times to ensure accuracy and consistency. The percentage of inhibition was plotted against the concentration, and the IC50 values were calculated using this data.

$$\text{DPPH \% Inhibition} = (\text{A control} - \text{A sample} / \text{A control}) \times 100$$

Determination of antioxidant Potential by ABTS assay

The ABTS scavenging activity of the MSC formulation was evaluated and compared to that of the reference chemical, gallic acid. (15, 16) The experimental approach consisted of preparing a 7.4 mM ABTS solution by using a 2.5 mM potassium persulphate solution. The solution was then placed in a controlled environment with limited light exposure for 12 to 18 hours at room temperature, leading to a stable oxidative state. The solution was adjusted to an absorbance value of 0.70 by combining 1 ml of ABTS solution with 60 ml of methanol. Subsequently, 750 µl of MSC samples at various concentrations ranging from 10 to 100 µg/mL were mixed with a 250 µl solution of ABTS. The solution was incubated for 6 minutes in darkness. Measurements of absorbance were taken for every sample and standard (Gallic acid). Absorbance at a wavelength of 734 nm was measured to ascertain the percentage of ABTS scavenging, as determined by the given formula.

$$\text{ABTS scavenging (\%)} = (\text{A control} - \text{A sample} / \text{A control}) \times 100$$

Determination of antioxidant Potential by Hydrogen peroxide radical scavenging assay

A solution containing 2 millimoles per litre of hydrogen peroxide was produced in a phosphate buffer with a concentration of 50 millimoles per litre and a pH of 7.4, 0.1 mL portions of the test sample MSC, with concentrations ranging from 10-100 µg/mL, were added to test tubes. The contents were then adjusted to 0.4 mL using 50 mM phosphate buffer at pH 7.4. Following the addition of 0.6 mL of hydrogen peroxide solution, the tubes were vigorously mixed and the absorbance at 230 nm was measured 10 minutes later relative to a blank sample. BHA was employed as a positive control. (17) The percentage of inhibition for the test and standard has been calculated and recorded. The % radical scavenging activity of the MSC and BHA was estimated using the following formula:

Hydrogen peroxide radical scavenging (%) = (A control – A sample / A control0 × 100

Determination of antioxidant Potential by Nitric oxide radical scavenging assay

The test sample MSC is diluted in a range of concentrations, from 10−100 µg/mL, along with the standard ascorbic acid. (18) A solution of the Griess reagent was prepared by mixing equal volumes of two solutions. The first solution contained a 1% concentration of sulphanilamide in 2.5% phosphoric acid, while the second solution contained a 0.1% concentration of naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid. The mixture was prepared right before it was used. A 0.5 mL volume of a solution containing 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of different concentrations (ranging from 10−100 µg/mL) of the test medication. The mixture was incubated at a temperature of 25°C for a duration of 180 minutes. The MSC test sample was mixed with an equal amount of freshly prepared Griess reagent. Control samples without MSCs, but with the same amount of buffer, were created using a similar procedure as the test samples. The absorbance measurement was performed at a wavelength of 546 nm. Ascorbic acid was used as the positive control. The calculation and documentation of the inhibition percentage for the MSC and standard were completed. The proportion of radical scavenging activity was determined using a specific formula:

NO radical scavenging (%) = (A control – A sample / A control) ×100

Determination of α-Glucosidase Inhibitory Activity

The *in vitro* α-glucosidase inhibitory activity was performed using the approach described by Pistia-Brueggeman and Hollingsworth et al. (19) The MSC test formulation was prepared using a volume of 50 µL at various doses ranging from 100 to 500 µg/ml. The sample was dissolved in dimethyl sulfoxide and subsequently blended with a solution of 10 µL of α-glucosidase (maltase) at a concentration of 1 U/mL and 125 µL of 0.1 M phosphate buffer with a pH of 6.8. The solution was subjected to incubation for a period of twenty minutes at a temperature of 37 °C. The reaction was started by adding a solution containing 20 µL of pNPG (4-Nitrophenyl-β-D-glucopyranoside) at a concentration of 1 M, which acted as the substrate. The combination was subsequently incubated for a period of 30 minutes. 50 µL of a 0.1 N solution of Na2CO3 was added to stop the reaction. The optical density was measured at a wavelength of 405 nm using a spectrophotometer. Acarbose was used as a positive control to evaluate its inhibitory effects on α-amylase. The IC_{50} values for the test sample (MSC) and acarbose have been determined by calculating the dose-response curve.

Percentage inhibition= [(average A 405 control − average A 405 test sample (MSC)) /average A 405 control] × 100.

Determination of α-Amylase Inhibitory Activity

The α-amylase inhibitory activity was conducted using the method outlined by Kifle et al. (20) The MSC formulation was dissolved in a buffer solution with specific concentrations of NaCl and Na2HPO4, creating an environment with a pH of 6.9. The method resulted in the creation of various concentrations of the chemical, ranging from 100 to 500 µg/ml. A solution of α-amylase with a concentration of 2 units/ml was combined with a test sample (MSC) in a volume of 200 µl. The mixture was incubated at a temperature of 30°C for approximately 10 minutes. Afterward, 200 µl of a one percent starch solution was added to each tube, and it was incubated for approximately three minutes. The reaction was completed by adding 200 µl of DNSA reagent and heating it in a water bath maintained at 85°C for approximately ten minutes. Later, the mixture was allowed to cool to the surrounding temperature and then mixed with 5 ml of distilled water. The absorbance of the solution was measured at a wavelength of 540 nm using a UV-visible spectrophotometer. A control sample was created by replacing the test sample (MSC) with 200 µl of a buffer solution, resulting in an enzyme activity of 100%. A control group was established by using a blank solution that contained the test drug at various concentrations, without the presence of the enzyme solution. A control sample was prepared using acarbose, and the reaction was carried out in the same manner as the test sample (MSC) as previously described. The measurement of alpha-amylase inhibition is represented as a percentage and is computed using the following equation.

Inhibition (%) = [(Ac - Acb) - (As - Asb) / (Ac - Acb)] × 100.

In this formula, Ac represents the absorbance of the control, Acb represents the absorbance of the control blank, As represents the absorbance of the test sample, and Asb represents the absorbance of the test sample blank.

Statistical Analysis

The data are presented as the means ± standard deviation (SD). The statistical significance and differences were assessed using one-way analysis of variance (ANOVA), followed by Dunnett's t-test.

Results

Antioxidant potential of *Mega Sanjeevi Chooranam* against DPPH and ABTS radicals

The study formulation MSC was tested for its radical scavenging capacity against DPPH and ABTS, and the results were compared to those of ascorbic and gallic acid. The inhibition percentage against the corresponding radical was represented at the same dose (10-100 µg/ml). The formulation MSC demonstrates a noteworthy scavenging ability against the ABTS radical, reaching a maximum of 44.22±7.12%. This value is lower than the 94.96±1.26% exhibited by the standard gallic acid (Table 1). In Table 1, the IC_{50} values for the MSC and standard gallic acid were determined to be 110.1±18.6 µg/ml and 28.99 ± 3.58 µg/ml, respectively. The MSC formulation showed significant quenching of the DPPH radical, with inhibition percentages ranging from 17.96± 13.6 to 38.27±9.01% when compared to the standard ascorbic acid, which had inhibition percentages ranging from 30.23±15.29 to 91.75±10.82%. In Table 1, the IC_{50} values for the MSC and standard ascorbic acid were determined to be 144.4±36.8 µg/ml and 33.65±13.34µg/ml, respectively.

Table 1: DPPH and ABTS radical inhibitory activities of the *Mega Sanjeevi Chooranam* and standard

Concentration	Percentage (%) inhibition on DPPH radical scavenging assay		Percentage (%) inhibition on ABTS radical scavenging assay	
	Mega Sanjeevi Chooranam	Ascorbic Acid	*Mega Sanjeevi Chooranam*	Gallic Acid
10 µg/ml	17.96 ± 13.6	30.23 ± 15.29	4.494 ± 1.62	26.73 ± 2.95
20 µg/ml	20.72 ± 14.57	41.31 ± 3.67	12.84 ± 2.02	49.29 ± 3.86
40 µg/ml	26.8 ± 11.14	56.59 ± 11.93	23.36 ± 2.66	62.27 ± 2.62
60 µg/ml	30.82 ± 11.87	69.6 ± 7.88	30.29 ± 3.26	76.94 ± 0.83
80 µg/ml	35.44 ± 9.96	81.75 ± 5.12	39.31 ± 8.91	83.84 ± 2.02
100 µg/ml	38.27 ± 9.01	91.75 ± 10.82	44.22 ± 7.12	94.96 ± 1.26
IC_{50} µg/ml	144.4 ± 36.8	33.65 ± 13.34	110.1 ± 18.6	28.99 ± 3.58

The mean ± standard error of the mean (SEM) is reported for each value of percent inhibition of DPPH and ABTS radicals with a sample size (n) of 3. The value of IC_{50} indicates the half-maximal inhibitory concentration.

Antioxidant potential of *Mega Sanjeevi Chooranam* against H_2O_2 and NO radicals

The radical scavenging capacity of the study formulation MSC was assessed against H_2O_2 and NO, and the findings were compared to those of butylated hydroxy anisole (BHA) and ascorbic acid. The MSC formulation has a significant capacity to scavenge the H_2O_2 radical, with a maximum inhibition of 31.48±7.90%, in comparison with BHA which reveals a higher percentage inhibition of 92.11±8.45% (Table 2). The IC_{50} values for the MSC and standard BHA were measured as 172.7±39.3µg/ml and 38.38 ± 19.17 µg/ml, respectively. The MSC formulation exhibited notable suppression of the NO radical, with inhibition percentages ranging from 6.00 ±5.95 to 24.03±7.24% in comparison to the standard ascorbic acid, which had inhibition percentages ranging from 10.53±3.90 to 84.81 ± 1.76%. The IC_{50} values for the MSC and standard ascorbic acid were measured to be 238.4±58.56 µg/ml and 55.66±3.20µg/ml, respectively, as shown in Table 2.

Table 2: Hydrogen peroxide and Nitric oxide radical inhibitory activities of the *Mega Sanjeevi Chooranam* and standard

Concentration	Percentage (%) inhibition on H_2O_2 radical scavenging assay		Percentage (%) inhibition on NO radical scavenging assay	
	Mega Sanjeevi Chooranam	BHA	*Mega Sanjeevi Chooranam*	Ascorbic Acid
10 µg/ml	7.798 ± 8.16	29.15 ± 13.79	6.003 ± 5.95	10.53 ± 3.90
20 µg/ml	12.97 ± 8.01	40.94 ± 14.6	9.445 ± 6.81	26.46 ± 5.05
40 µg/ml	18.1 ± 9.97	51.54 ± 9.70	14.23 ± 8.47	38.82 ± 2.56
60 µg/ml	23.3 ± 9.22	59.23 ± 14.93	17.3 ± 8.24	46.93 ± 1.61
80 µg/ml	26.66 ± 8.09	76.1 ± 13.78	20.16 ± 8.37	73.09 ± 2.22
100 µg/ml	31.48 ± 7.90	92.11 ± 8.45	24.03 ± 7.24	84.81 ± 1.76
IC_{50} µg/ml	172.7 ± 39.3	38.38 ± 19.17	238.4 ± 58.56	55.66 ± 3.20

The mean ± standard error of the mean (SEM) is reported for each value of percent inhibition of H_2O_2 and NO radicals with a sample size (n) of 3. The value of IC_{50} indicates the half-maximal inhibitory concentration.

Inhibitory effect of *Megasanjeevi Chooranam* against α-amylase and α-glucosidase enzymes

In the current investigation, the efficacy of the formulation MSC and standard acarbose was assessed in terms of their inhibitory potential on α-amylase and α-amylase enzyme. The inhibitory activity ranges between 22.14 ± 3.78 % to 73.44 ± 6.87 %, were observed at the concentration varying from 100-500 µg/ml (Table 3). The half-maximal inhibitory concentration (IC_{50}) of MSC was determined to be 299.4 ± 27.27 µg/ml (Table 3). In comparison with the standard drug acarbose which exhibit the percentage inhibition of 94.67 ± 3.64 % with an IC_{50} value of 23.47±2.80µg/ml (Table 3).

In relation to the α-glucosidase enzyme inhibitory activity, the test formulation MSC demonstrated inhibitory percentage between 22.08 ± 13.39 to 48.89 ± 13.38% at the concentration ranging from 100 to 500 µg/ml in comparison with the standard acarbose which exhibit the percentage inhibition of 93.84 ± 0.54 %. The IC_{50} values for the MSC and standard acarbose was found to be 402.2 ± 86µg/ml and 29.03 ± 21.46 µg/ml, respectively, as shown in Table 3.

Table 3: α-glucosidase and α-amylase enzyme inhibitory activities of the *Mega Sanjeevi Chooranam* and standard acarbose

Concentration	Percentage (%) inhibition on α-Glucosidase enzyme		Percentage (%) inhibition on α-Amylase enzyme	
	Mega Sanjeevi Chooranam	Acarbose	*Mega Sanjeevi Chooranam*	Acarbose
100 µg/ml	22.08 ± 13.39	35.4 ± 1.60	22.14 ± 3.78	25.49 ± 3.62
200 µg/ml	28.4 ± 11.03	57.26 ± 0.49	43.04 ± 2.53	59.27 ± 0.64
300 µg/ml	34.22 ± 12.03	74.39 ± 3.38	54.7 ± 1.41	69.55 ± 2.14
400 µg/ml	41.91 ± 12.24	80.59 ± 0.70	64.17 ± 1.33	84.06 ± 0.48
500 µg/ml	48.89 ± 13.38	93.84 ± 0.54	73.44 ± 6.87	94.67 ± 3.64
IC_{50} µg/ml	402.2 ± 86	29.03 ± 21.46	299.4 ± 27.27	23.47 ± 2.80

The mean ± standard error of the mean (SEM) is reported for each value of percent inhibition of α-glucosidase and α-amylase with a sample size (n) of 3. The value of IC_{50} indicates the half-maximal inhibitory concentration.

Discussion

Oxidative stress has become a topic of great interest in recent years, as it has a significant impact on human health, especially its connection to diabetes. Oxidative stress arises from an imbalance between the generation of reactive oxygen species (ROS) and the body's capacity to counteract them with antioxidants. ROS, produced during normal cellular metabolism, include free radicals and other highly reactive molecules. Nevertheless, if the body's antioxidants cannot keep up with the excessive production, it leads to oxidative stress. (21, 22) The implications of oxidative stress in diabetes are extensive and varied. The dysfunction and apoptosis of beta-cells are a result of the harmful impact of reactive oxygen species (ROS), which further impairs the production and secretion of insulin. Oxidative stress can have a negative impact on insulin effectiveness, making it harder for cells to absorb glucose. (23) It is widely recognised that natural products play a crucial role in the treatment of diabetes (24) effectively managing the condition and reducing the risk of complications. (25)

In order to evaluate the antioxidant capacity of various components, such as herbal therapeutics, dietary elements, and pharmaceuticals, the ABTS radical scavenging test is a technique that is often utilised at the present day. (26) It offers a fast and dependable method to evaluate the possible health advantages of antioxidants, therefore helping to studies in the fields of nutrition, pharmacology, and environmental science. The ABTS assay's flexibility enables its application in a broad spectrum of research, ranging from assessing the antioxidant characteristics of specific substances to examining intricate combinations. (27) Outcome of our study evidence that MSC exhibits a remarkable ability to scavenge the ABTS radical, with a maximum of 44.22±7.12%. The value is lower compared to the standard gallic acid, which exhibited a range of 94.96±1.26%. The IC50 values for the MSC and standard gallic acid, which were found to be 110.1±18.6 µg/ml and 28.99 ± 3.58 µg/ml, respectively.

The DPPH radical scavenging activity test plays a crucial role in scientific research and the development of herbal-based medicinal medicines. The herbs in question exhibit a remarkable ability to scavenge DPPH, indicating their strong antioxidant properties. This finding further reinforces the potential of these herbs in protecting against oxidative stress-induced damage. (28) Results of our study indicates that the MSC exhibited notable reduction of the DPPH radical, with inhibition percentages ranging from 17.96± 13.6 to 38.27±9.01% in comparison to the standard ascorbic acid, which displayed inhibition percentages ranging from 30.23±15.29 to 91.75±10.82%. Table 1 displays the IC50 values for the MSC and standard ascorbic acid, which were found to be 144.4±36.8 µg/ml and 33.65±13.34µg/ml, respectively.

Hydrogen peroxide is a reactive oxygen species that may cause oxidative damage to cells and biomolecules, which can contribute to a variety of illnesses and the ageing process. By determining the scavenging activity of the formulation against hydrogen peroxide, researchers are able to estimate the usefulness of these herbal preparations in limiting the damage that is caused by oxidative stress. (29) The significance of this lies in the fact that antioxidants found in herbs have the ability to reduce the negative effects of free radicals, hence boosting health and maybe delivering therapeutic advantages. Our investigation found that the MSC formulation effectively neutralises hydrogen peroxide radicals, with a maximum inhibition rate of 31.48±7.90%. In comparison, BHA has a greater percentage inhibition of 92.11±8.45%. The IC50 values for MSC and conventional BHA were 172.7 ± 39.3 µg/ml and 38.38 ± 19.17 µg/ml.

The nitric oxide scavenging test assesses the capacity of the herbal formulations to counteract nitric oxide (NO), a highly reactive nitrogen molecule that can induce oxidative damage. The primary importance of this test is to quantify the efficacy of herbs in preventing cellular damage generated by NO. This test aids in validating the antioxidant potential and therapeutic advantages of herbal treatments by assessing their capacity to scavenge NO. (30) Outcome

of our study advocates that MSC formulation exhibited dose dependent suppression of the NO radical, with inhibition percentages ranging from 6.00 ±5.95 to 24.03±7.24% in comparison to the standard ascorbic acid, which had inhibition percentages ranging from 10.53±3.90 to 84.81 ± 1.76%. The IC_{50} values for the MSC and standard ascorbic acid were measured to be 238.4±58.56 µg/ml and 55.66±3.20µg/ml.

There are multiple factors involved in the development of type 2 diabetes, such as genetics, environment, lifestyle, and obesity. Research has shown that an unhealthy diet significantly increases the risk of developing diabetes. There is a sudden rise in blood glucose levels in individuals with type 2 diabetes, resulting from the breakdown of starch by pancreatic α-amylase and the absorption of glucose by intestinal α-glucosidases. (31) A highly effective approach to managing type 2 diabetes involves strongly inhibiting intestinal α-glucosidases and mildly inhibiting pancreatic α-amylase. (32) α-Amylase can be found in both salivary and pancreatic secretion. Its main role is to break down large malto-oligosaccharides into maltose, which can then be used by intestinal glucosidase as a substrate. (33)

Several studies have indicated a correlation between the antidiabetic and antioxidant properties of medicinal plants.(34) Reducing post-prandial hyperglycemia with natural products offers a therapeutic alternative for diabetes treatment. This is achieved by slowing down the absorption of glucose through the inhibition of enzymes responsible for breaking down carbohydrates in the digestive tract. (35) Several studies have emphasised the potential benefits of bioactive compounds with antioxidant activity in managing diabetes mellitus by inhibiting certain enzymes. (36)

Glucosidase inhibitors are commonly given to people with diabetes to reduce the development of postprandial hyperglycemia. This condition is caused by the enzymatic breakdown of starch in the small intestine.(37) In our study the test formulation MSC showed inhibitory percentages ranging from 22.08 ± 13.39 to 48.89 ± 13.38% in relation to the α-glucosidase enzyme inhibitory activity, in comparison, the standard acarbose exhibited a much higher percentage inhibition of 93.84 ± 0.54%. The IC_{50} values for the MSC and standard acarbose were determined to be 402.2 ± 86µg/ml when compared to standard acarbose (29.03 ± 21.46 µg/ml).

α-amylase is a crucial enzyme that plays a vital role in breaking down starch and determining the amount of glucose that is released. It is suggested to incorporate therapeutics that helps to regulate postprandial hyperglycemia. These compounds have the ability to slow down the digestion of starch by inhibiting the activity of α-amylase. (38) It was evident from our study that the formulation MSC exhibit highest inhibition of about 73.44 ± 6.87 % against the enzyme alpha amylase in comparison with standard acarbose which emphasise 94.67 ± 3.64 % inhibition. The IC_{50} values for the MSC was found to be 299.4 ± 27.27 µg/ml when compared to standard acarbose (23.47±2.80µg/ml).

Conclusion

Diabetes mellitus has long been recognised as a significant factor impacting the financial well-being of individuals, their loved ones, and the broader community. In addition, uncontrolled diabetes can result in severe long-term complications, including vision loss, kidney damage, and heart problems. Efforts are being made to address this issue by conducting research on novel antidiabetic agents. Traditional medicines have gained attention due to the negative impacts of modern therapies. In addition, the use of herbal preparations in combination with standard drugs has become increasingly common in modern therapies. Every herb possesses unique active ingredients that have the potential to reduce blood sugar levels and manage the various complications associated with diabetes. The *Mega Sanjeevi Chooranam* demonstrated the most potent antioxidant activity and effectively suppressed the α-amylase and α-glucosidase enzymes in a dose-dependent manner. Thus, it is imperative to conduct additional research on the MSC like preparations to enhance its separation, purification, and material structure identification. Additionally, it is crucial to delve deeper into its mechanism of action and explore the potential synergy among various bioactive compounds.

Conflict of Interest: The authors have no conflicts of interest regarding this investigation.

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