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# ANTIOXIDANT ACTIVITIES OF SIDDHA POLYHERBAL FORMULATION *MADHUMEGA CHOORANAM* (MMC) USING IN VITRO ASSAY MODELS

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## ABSTRACT

**Background:** Siddha system of medicine has its origin from Dravidian civilization is being in practice in southern parts of India, northern Sri Lanka, Malaysia and also in Tamil speaking parts of the world. *Madhumega chooranam*(MMC) is the Siddha polyherbal formulation which is being used traditionally for the control of blood sugar containing seven herbs viz., *Murraya koenigii, Terminalia chebula, Emblica officinalis, Tinospora cordifolia, Syzygium cumini, Cyperus rotundus and Phyllanthus niruri*. In diabetes mellitus, persistent hyperglycemia causes increased production of free radicals, mainly reactive oxygen species (ROS), from auto-oxidation and glycosylation of glucose and protein respectively. Hence there is an imbalance in the antioxidant level which causes oxidative stress, which plays a vital role in presenting vascular complications. The present study was carried out to investigate the *in-vitro* anti-oxidant activity of Madhumega chooranam. **Methods:** *Madhumega chooranam* (MMC) was studied for in-vitro antioxidant activity along with phenolic content to evaluate the radical quenching potential. In-vitro assays like DPPH radical scavenging activity, pro-oxidant or reducing power effect, total antioxidant activity, superoxide radical scavenging activities were carried out and compared with the standard ascorbic acid. **Results:** The drug MMC had shown 61.57% inhibition at 140µg for DPPH free radical scavenging activity against 68% for standard ascorbic acid ($IC_{50}$ for MMC is 92.4 µg/ml and 63.0 µg/ml for standard). $IC_{50}$ for pro oxidant activity of MMC was 88.45µg/ml while the standard was 92.7 µg/ml. $IC_{50}$ for total anti-oxidant activity of MMC was 27.82 µg/ml, (standard 100.75 µg/ml). $IC_{50}$ for super oxide

radical scavenging activity of MMC was 70.70µg/ml (standard, 88.00 µg/ml). **Conclusion:** This study has validated the use of the anti-diabetic Siddha polyherbal formulation Madhumega chooranam as a potential anti-oxidant.

**KEYWORDS:** Siddha, polyherbal drug, madhumega chooranam, antioxidant activity.

## INTRODUCTION

Siddha system of medicine has its origin from Dravidian civilization is being in practice in southern parts of India, northern Sri Lanka, Malaysia and also in Tamil speaking parts of the world. *Madhumega chooranam*(MMC) is the Siddha polyherbal formulation which is being used traditionally for the control of blood sugar containing seven herbs viz., *Murraya koenigii, Terminalia chebula, Emblica officinalis, Tinospora cordifolia, Syzygium cumini, Cyperus rotundus and Phyllanthus niruri*.[1,2] In diabetes mellitus, persistent hyperglycemia causes increased production of free radicals, mainly reactive oxygen species (ROS), from auto-oxidation and glycosylation of glucose and protein respectively. The level of ROS increases thereby an imbalance in the antioxidant level which causes oxidative stress, which plays a vital role in presenting vascular complications. An equilibrium should be sustained in the body for free radicals and anti-oxidants to maintain healthy tissue structure and function. Anti-oxidants mediate their defensive mechanism by quenching the free radicals directly and thus prevent the cellular damage.[3]

The non-enzymatic phenolic components, flavanoids, ascorbic acid etc., act by various mechanism like free radical scavenging, reducing activity, formation of pro-oxidant metal complexes and quenching of singlet oxygen. ROS immediately reacts and oxidizes bio-molecules including carbohydrates, proteins, lipids and DNA.[4] The circulating excess free radicals in the body oxidize the low density lipoproteins (LDL), make them potentially harmful and also accelerate aging process leading to various pathologies like diabetes mellitus, stroke, rheumatoid arthritis, parkinson's disease, alzheimer's disease and cancer.[5] The present study was carried out to investigate the *in-vitro* anti-oxidant activity of *Madhumega chooranam*.

## MATERIALS AND METHODS

**Chemicals:** All the chemicals used were of analytical grade and purchased from M/s.Himedia laboratories, India.

**Formulation:** The Siddha drug, *Madhumega chooranam* was obtained from M/s.Ulaga Tamizh Maruthuva Kazhagam, Tirunelveli and stored at room temperature.

**Evaluation of *in vitro* antioxidant activity**

**DPPH Radical Scavenging Assay**

0.2 mg/ml of MMC aqueous extract was used for diphenyl-picrylhydrazyl (DPPH) radical assay, with mild modifications and a final concentration range of 20-140 µg/ml was used for the assay. The sample was made up to 1 ml with 95% methanol followed by 1 ml of 0.2 mM DPPH, incubated in dark for 30 minutes. At 515 nm, the purple colour developed was read spectrophotometrically (Shimadzu UV-VIS 1800 spectrophotometer; Shimadzu Corporation, Kyoto, Japan). The standard was the ascorbic acid and 95% methanol was the blank. A mixture of 95% methanol and DPPH was used as a control.[6,7] The percentage of Inhibition was calculated using the formula.

% I = (C – E) / C

Whereas % I=% Inhibition; C=Absorbance of control; E=Absorbance of extract.

**Determination of pro-oxidant**

To determine the reducing power (Pro- oxidant) of the drug. The principle in which the substances which have reaction potential which reacts with potassium ferricyanide (Fe3+) then reacts with ferric chloride which forms ferric-ferrous cyanide and has an absorption maximum at 700 nm. The chemicals were prepared by dissolving 1g of Potassium ferricyanide in 100 ml of distilled water (1% w/v). Phosphate buffer (0.2 M, Ph-6.6) was prepared by dissolving 2.75 g of sodium dihydrogen phosphate and 5.365g of disodium hydrogen phosphate in 100 ml of distilled water. 10 g of Trichloro acetic acid (10%) was dissolved in 100 ml of distilled water and 0.1 g of Ferric chloride in 100 ml of distilled water. Various concentration of the drug and the standard quercitin were mixed with 2.5 ml of phosphate buffer and potassium ferricyanide and was kept for 20 minutes in the water bath at 50°C. After cooling, 2.5 ml of 10% Trichloro acetic acid was added and centrifuged at 3000rpm for 10 minutes. 2.5ml of supernatant was mixed with equal volume of distilled water and 0.5% ferric chloride solution. The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples, where the saturation point reaches the concentration was taken as 50% inhibition by the drug.[8]

**Determination of total antioxidant capacity**

The total antioxidant activity of the drug was determined by reduction of Mo (VI) to Mo (V) by the samples and the consequent formation of a green coloured Mo (V) / Phosphate complex. The reagents were prepared with 28mM of sodium phosphate, dissolving 84 mg of sodium phosphate in 25 ml of distilled water. 4mM of Ammonium molybdate was prepared by dissolving 124 mg of ammonium molybdate in 25 ml of distilled water and 0.6 mM of sulphuric acid. 100 µg of the drug in 0.3 ml of distilled water was added to 3 ml of molybdate reagent containing 0.6 mM sulphuric acid, 28 mM of sodium phosphate and 4 mM of ammonium molybdate. The tubes were incubated at 95°C for 90 minutes, then cooled to room temperature and the absorbance was measured at 695 nm with the spectrophotometer. The results were expressed as equivalents of ascorbic acid.[9]

**Scavenging of superoxide radical by alkaline DMSO method**

The superoxide scavenging effect of the drug was studied using the alkaline DMSO method. The Reagents were prepared to 0.1 ml of NBT was prepared by dissolving 1 mg/ml of NBT ( Nitro blue Tetra Zolium) in 1 ml of DMSO (Dimethyl Sulfoxide). 1 ml of alkaline DMSO was prepared by dissolving 5 mM of sodium hydroxide in 1 ml of DMSO. The samples were dissolved in DMSO in various concentration were added with 0.1 ml of NBT (1 mg / ml of DMSO) and 1 ml of alkaline DMSO (5 mM of sodium hydroxide / ml of DMSO). Then the absorbance was measured at 560 nm using spectrophotometer.[10]

**Percentage of antioxidant activity was calculated by the formula**

$$\textbf{Percentage of Inhibition} = \frac{\textbf{(Absorbance of control - Absorbance of test)}}{\textbf{(Absorbance of control)}} \times 100$$

**Statistical Analysis**

Analysis was performed as per standard protocol. Analysis was performed in duplicates. The results were expressed in mean ± SEM and calculated IC50 values using GraphPad PRISM Software (Version 4).

**RESULTS**

The percentage inhibition of MMC for DPPH radical scavenging activity was in dose dependent manner and its $IC_{50}$ is 92.4 µg/ml compared to the standard, 63.0 µg/ml(Fig1). The percentage inhibition of MMC for pro-oxidant effect was in dose dependent manner and its $IC_{50}$ is 88.45µg/ml while the standard was 92.7µg/ml (Fig2). The total anti-oxidant activity of

MMC was much ($IC_{50}$ =27.82 µg/ml) much better than the standard ($IC_{50}$ =100.75 µg/ml) (Fig3). The superoxide radical scavenging of the drug ($IC_{50}$ =70.70µg/ml) is better than the standard ($IC_{50}$ =88.00 µg/ml) (Fig4).

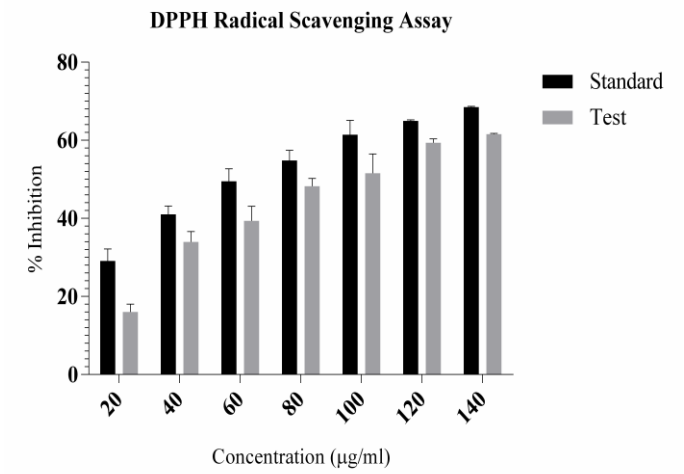


**Fig. 1. Determination of DPPH radical scavenging activity of MMC with the standard, ascorbic acid.**

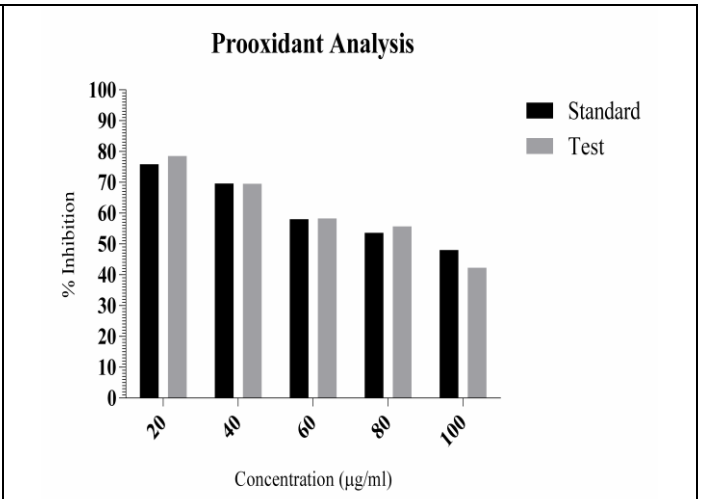


**Fig. 2. Determination of prooxidant effect of the drug, MMC with the standard, ascorbic acid.**

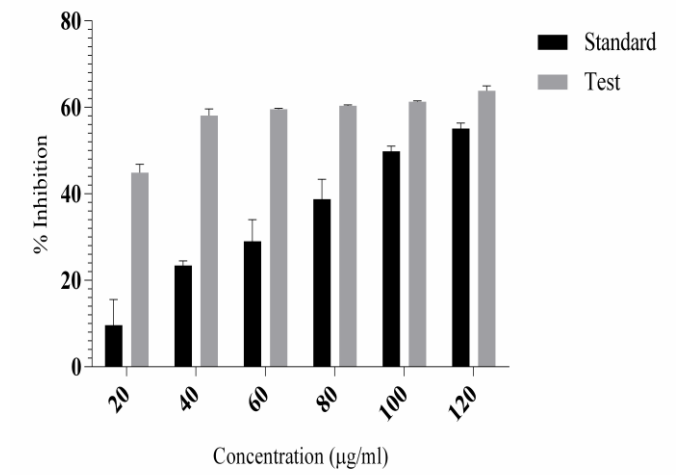


**Fig. 3. Determination of total antioxidant capacity of the drug, MMC with the standard, ascorbic acid.**

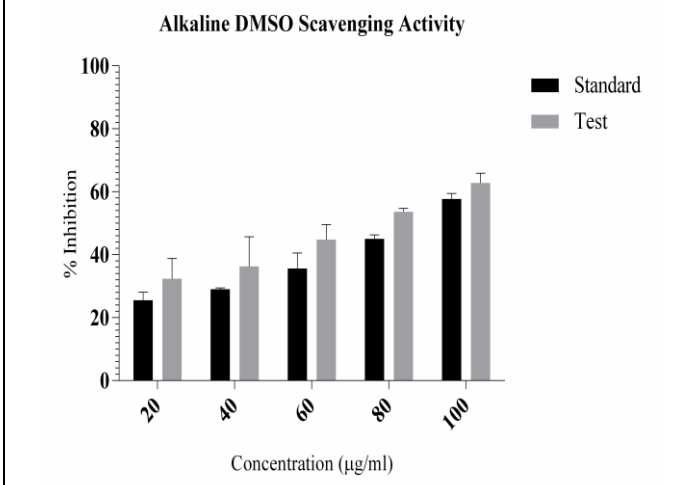


**Fig. 4. Determination of super oxide radical scavenging activity of the drug, MMC with the standard.**

## DISCUSSION

Free radicals are chemical structures which have one or more unpaired electrons, which are highly unstable and in order to attain stability they extract electrons from other molecules and inturn damage them.

These radicals are continuously produced in the human body and are regulated by endogenous anti-oxidant enzyme system. The various enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx) etc., and non-enzymatic anti-oxidants like

ascorbic acid, flavanoids, phenolic compounds, etc act by one or more mechanisms like free radical scavenging, quenching of singlet oxygen or formation of pro-oxidant metal complexes. In order to prevent the ill effects either the body's natural anti-oxidants is enhanced or should be supplemented by proven natural anti-oxidants from herbal sources.[11] The anti-oxidant activity of *Madhumega chooranam* was investigated using various *in vitro* methods for its scavenging activity.

From the result we observe a dose dependent DPPH radical scavenging activity of the Siddha polyherbal formulation *Madhumega chooranam* (MMC) with the $IC_{50}$ is 92.4 µg/ml when compared to the standard, 63.0 µg/ml. The pro-oxidant effect, total anti-oxidant activity and the superoxide radical scavenging activity of MMC was better than the standard. Flavanoids and phenolic compounds in MMC could be the reason for possessing the anti-oxidant activity.

Superoxide radical is a precursor, which is very harmful to cellular components. It is produced *in-vivo* through dismutation reaction resulting in the formation of hydrogen peroxide.[12] Anti-oxidant activity of the compounds was based on their ability to donate hydrogen atoms to free radicals which play an important role in absorbing and neutralizing free radicals, decomposing peroxides or quenching singlet and triplet oxygen.[13] The Siddha drug MMC was found to have anti-oxidant activity at different levels based on the various methods tested. Further detailed studies will be conducted to deduce the antioxidant activity and mechanisms *in-vivo.*

## CONCLUSION

From this study it was obvious that the total phenol content and flavonoid in *Madhumega chooranam* could be the major contributors to the anti-oxidant activity.

## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## ACKNOWLEDGEMENT

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