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In-Vitro Evaluation Of Antioxidant Activity Of Classical Siddha Drug-Malattu Karpam

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Abstract

Aim and Objectives

Siddha system of medicine is a treasure dedicated to the world by Siddhars. Siddha system is one of the traditional medical sciences which describe the lifestyle methods for living a healthy life along with medicine. The word Kaya Karpam means (Kayam-body, Karpam-able, competent) to make our body competent and youthful. The Kaya Karpam division also encompasses our test drug Malattu Karpam in that manner. This polyherbal formulation composed of 3 herbal ingredients is indicated for the treatment of infertility in siddha classical literature "Theriyar Yemagha Venba" (Pg no 70,71). So, this paper is aimed to evaluate in-vitro antioxidant activities of the test drug.

Materials and Methods

The antioxidant activity of Malattu Karpam was evaluated through in-vitro assays such as DPPH (2.2-diphenyl-1-picrylhydrazyl), Nitric Oxide radical scavenging assay, Total Iron Reducing power assay, Superoxide Radical Scavenging activity using standard methods**.**

Results

The results of in-vitro antioxidant assays show significant free radical scavenging properties of Malattu Karpam**.**

Conclusion

The study concluded that test drug Malattu Karpam has potential antioxidant activity.

Keywords: Malattu Karpam, Antioxidant, Kaya Karpam, Infertility.

Introduction

Siddha System of medicine is a part of Tamil culture that originated in South India and is considered to be one of the India's oldest system of medicine. It is based on the combination of ancient medicinal practices and spiritual disciplines. The word "Siddha" comes from the word 'Siddhi" which means "achievement or an object to be attained.

The objective of Siddha medicine differs profoundly from that of other systems in one respect. The prevention and the cure of illness are the basic aim of all system of medicine whereas the Siddha system has in addition the transcendental motivation. (i.e.) immortality of the body using kaya Karpam drugs.

The siddha system of medicine categorized into two classes.

1. Internal medicine and
2. External medicine.

Among 32 Internal medicine, KARPAM is one among them and its shelf life period over centuries (rejuvenating medicine i.e. preparation used for rejuvenation).

Karpam: leaves, roots or some metallic and Uparasas are taken in Prescribed dose with Specific instructions over a period of time. The is of two types.

A. Those medicaments which have to be prepared daily Preparation of leaves, Roots etc.
B. Pre- Prepared medince medicaments; metallic and Uparasa preparation.

To have an idea of the Kaya karpam, we give a few names of manuscripts and printed books that deal about this Subject 1. Agathiyar karpam 2.Pulathiyar karpam 3.Sattamuni karpam 4.Macchamuni karpam.

There are about 108 karpams and methods available from the siddha literature. IN the group of Mooligai karpam, there are so in number starting from kadukai karpam ending in Natha vindu karpam. The remaining 48 karpams belong to metallic and mineral preparation. Apart from all, there is a vasi yoga karpam based on the science of breathing. The duration of taking the kalpam ranges from one mandalam to three mandalam as per the old age.Older the person the longer should be the duration of taking karpam.

As a general precaution the diet should be like light and agreeable the atmosphere should be peaceful with the association of vise people, while taking the karpam.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation reactions can form free radicals and these start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions.

Antioxidants are substances, which stop or delay oxidation of a substrate while present in minute amounts. Endogenous antioxidant defences are both non-enzymatic (e.g., Uric acid, glutathione, bilirubin, thiols, albumin and nutrional factors, including vitamins and phenols) and enzymatic (e.g., the superoxide dismutase's, the glutathione peroxidases (GSHPx), and catalase).In the normal subject the endogenous antioxidant defences balance the reactive oxygen species production, but for the above mentioned 1% daily leak. The important source of anti-oxidants is provided by nutrition, may belonging to phenol family.

Through the reduction of free radicals, antioxidant chemicals provided a protective mechanism against oxidative damage to cells. In the body, oxidation is a regular chemical process that occurs every day. Free radicals are very unstable and potentially harmful chemicals that arise when the natural oxidation process is disrupted.

Free radical's reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. A balance between free

radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues. Prolonged oxidative stress can result in permanent damage to vital organs in our body, which could eventually lead to chronic disorders such as heart disease, diabetes, cirrhosis, malaria, neurodegenerative diseases, AIDS, cancer and premature aging. It has been noted that about 95% of the pathologies observed in people above 35 years of age are associated with production and accumulation of free radicals.

In order to globalize Siddha medicines, first mechanism of action of the drug and their activities is needed to analyze and to standardize the drug. Hence this study is carried to analyze the activity of the drug.

Review of Literature

மலட்டுக் கற்ப மருந்து

மாயா மலட்டு வலிக்ககமதவல் சம்படிநீர்
மாயா மலட்டு மடலட்டு மாயா
பகரணமண் டாமலலாரு பங்கீடாய் நாளும்
பகரணமண் டாமலுண்டு பார்.

-தேரன் யமக வெண்பா

மூங்கில் வேர் –Bambusa vulgaris
கருவேல மர வேர் –Acacia nilotica
நாவல் மர வேர் –Syzygium cumini

Materials and Methods

A. Procurement of raw drugs

The Raw drugs for the preparation of Medicine Malattu Karpam, was Purchased from the reputed indigenous drug store at Parry's Corner, Chennai.

Raw drugs:

- Moongil vaer (*Bambusa vulgaris*) -1/2 palam (17.5g)
- Karuvaelam vaer (*Acacia nilotica*) - 1/2 palam (17.5g)
- Naval vaer (*Syzygium cumini*)- 1/2 palam (17.5g)

B. Identification and authentication

The raw drugs for the preparation of Malattu Karpam, was identified and authenticated by Dr. S. Sankaranarayanan, HOD, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamil Nadu. The test drug MALATTU KARPAM was prepared as per Theriyar Yemagha Venba.

C. Procedure

Purification

The roots are washed thoroughly with river water and then dried. It made into a fine powder using grinding motor and seived with the process called vasthirakayam.

Preparation

To the above mentioned drugs, add 3/4padi (1140ml) of drinking water into a vessel and boil it until it reduces to ¼ part of the total content of decoction. Add palm jaggery and cow butter of each ½ palam (17.5g) to the decoction and drink for 1 Mandalam (48 days) without touching upper and lower lips.

DPPH Radical scavenging assay:

0.2 mg/ml of drug was used for diphenyl-picrylhydrazyl (DPPH) radical assay, with some modifications, and a final concentration range of 20-100 µg/ml was used for the assay. The sample was made up to 1 ml with 95% methanol followed by which 1 ml of 0.2 mM DPPH was added and incubated in the dark for 30 min. The purple color developed was read spectrophotometrically at 515 nm. Ascorbic acid (20 µg-100 µg/ml) was used as standard and 95% methanol alone was used as blank. A reaction mixture with 95% methanol and DPPH alone was used as a control(1.591). The % Inhibition was calculated using the formula:

$$\% I = (C - E) / C \times 100$$

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

Nitric oxide radical inhibition assay

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be estimated using the Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. The reaction mixture (6 mL) contained sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (PBS, pH 7.4, 1 mL), and extract or standard (1 mL) in Dimethyl sulfoxide (DMSO) at various concentrations and it was incubated at 25 for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite ion was removed, sulphanilic acid reagent was added (0.33% w/v, 1 mL), mixed well, and allowed to stand for 5 min for completion of diazotization. Then, 1 mL of N-(1-Naphthyl)- ethylendiamine - dihydrochloride (NEDD) was added, mixed, and allowed to stand for 30 min in diffused light. A pink-colored chromophore was formed. The absorbance was measured at 540 nm. (CONTROL -1.323)

Total iron reducing power assay

A volume of 1 mL of the plant extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated at 50 for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added and the reaction mixture was centrifuged at 1000 rpm for 10 min. The upper 2.5 mL layer was mixed with 2.5 mL of deionized water and 0.5 mL of ferric chloride and thoroughly mixed. The absorbance was measured spectrophotometrically at 700 nm. A higher absorbance indicates a higher reducing power. (CONTROL-1.964)

Superoxide radical scavenging activity by alkaline DMSO method

In this method, superoxide radical is generated by the addition of sodium hydroxide to air saturated DMSO. The generated superoxide remains stable in solution and reduces nitroblue tetrazolium (NBT) into form azan dye at room temperature which can be measured at 560 nm. Briefly, 0.1ml of NBT (1 mg/ml) was added to the reaction mixture containg 1 ml of alkaline DMSO (1ml DMSO containing 5mM of NaoH in 0.1 ml water) and 0.3 ml of the extract in freshly distilled DMSO at various concentration, to give a final volume of 1.4mL. the absorbance was measured at 560nm.

Evaluation of Bioactive Constituents

Estimation of Total phenolic content:

Total phenolic content was determined using gallic acid as the reference standard. One milliliter of the sample (0.2-1 mg/mL) was added with 0.5 mL of Folin Ciocalteaue reagent and incubated at room temperature for 10 minutes. Furthermore, 2.5 mL of saturated Na2CO3 solution was added and incubated at room temperature for 30 minutes. The resultant color was measured at 750 nm versus a blank containing distilled water and Folin Ciocalteau reagent. CONTROL as 2.316.

Total phenolic content was expressed in gallic acid equivalents using the equation

$$T = C \times V / M$$

(T = Total Phenolic Content (mg/g) of extract as gallic acid equivalents,
C =Concentration of Gallic acid established from the calibration curve,
V = Volume of the extract solution in ml,
M = weight of extract in grams**).**

Determination of total flavonoid content

Total flavonoid content was determined with slight modifications using quercetin as the reference standard. One milliliter of sample (0.2-1 mg/mL) was added with 0.5 mL of 1.2% aluminum chloride in 10% methanol, 0.5 mL of 1M potassium acetate, and made up to 3 mL with distilled water. The mixture was incubated for 30 minutes in the dark, and the absorbance was read at 415 nm. Aluminum chloride without the sample alone served as the blank. Total flavonoid content was expressed in gram quercetin equivalents. CONTROL AS 1.145.

Results

Tabl 1 Estimation of Total phenolic content:

S.No	Drug/Std Concentration (µg/ml)	Std	Test 1	Test 2	Test 3
1	10	0.091	0.412	0.403	0.398
2	20	0.152	0.705	0.704	0.7
3	30	0.191	0.949	0.949	0.945
4	40	0.275	1.137	1.141	1.152
5	50	0.238	1.337	1.303	1.314

Total Phenolic Content expressed as 5.959±0.749 mg/g gallic acid equivalents

Table 2 Determination of total flavonoid content

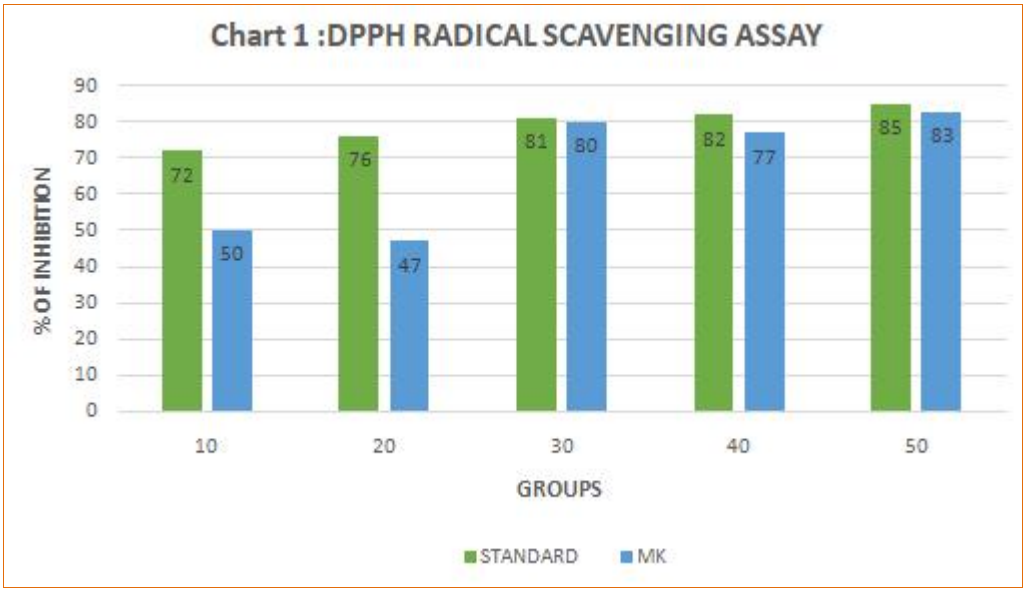
S.No	Drug/Std concentration (µg/ml)		Std	Test 1	Test 2	Test 3
1	10		0.071	0.129	0.121	0.125
2	20		0.085	0.182	0.187	0.186
3	30		0.068	0.197	0.203	0.204
4	40		0.071	0.317	0.358	0.349
5	50		0.073	0.387	0.387	0.384

The total Flavonoid Content is expressed as 6.154 ± 0.768 mg/g quercetin equivalents

DPPH Radical scavenging assay

Table 3

S.No	Drug Conc (µg/ml)	Std	% of inhibition	Test 1	% of inhibition
1	10	0.327	72	0.792	50
2	20	0.285	76	0.836	47
3	30	0.228	81	0.311	80
4	40	0.219	82	0.363	77
5	50	0.179	85	0.263	83

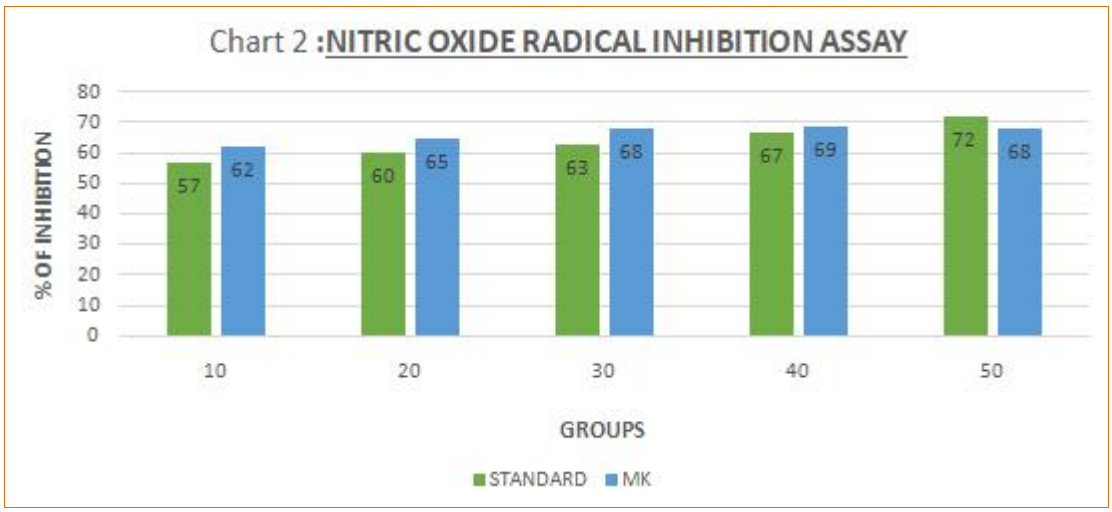


*** MK - Malattu Karpam**

Nitric oxide radical inhibition assay

Table 4

S.No	Drug conc (µg/ml)	Std	% of inhibition	Test 1	% of inhibition
1	10	0.825	57%	0.502	62
2	20	0.751	60%	0.459	65
3	30	0.694	63%	0.437	68
4	40	0.624	67%	0.436	69
5	50	0.526	72%	0.414	68

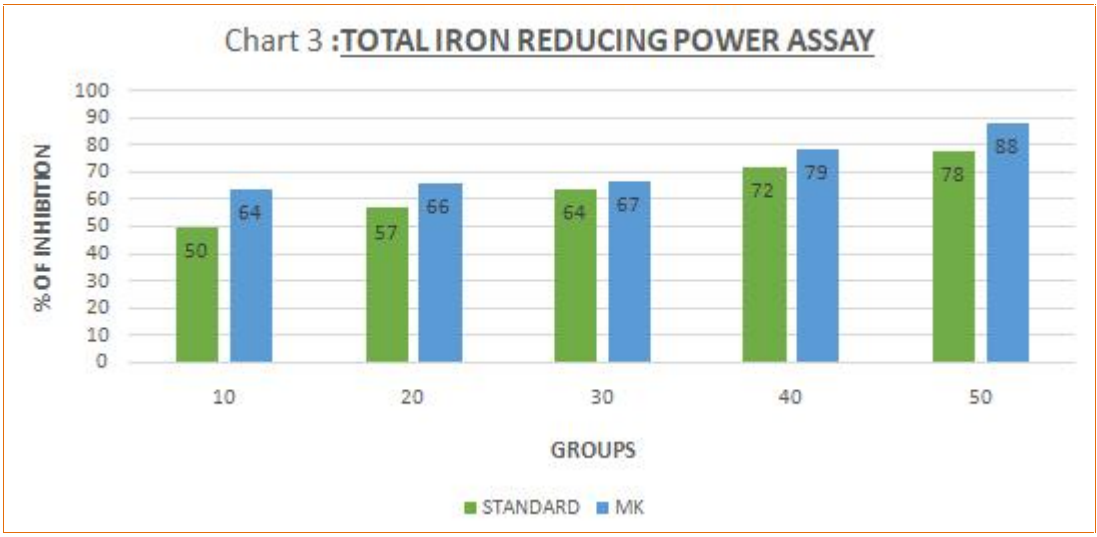


*** MK - Malattu Karpam**

Total iron reducing power assay

Table 5

S.No	Drug conc (µg/ml)	Std	% of inhibition	Test 1	% of inhibition
1	10	0.977	50.254	0.706	64
2	20	0.843	57.077	0.669	66
3	30	0.689	64.918	0.659	67
4	40	0.539	72.556	0.405	79
5	50	0.421	78.564	0.223	88

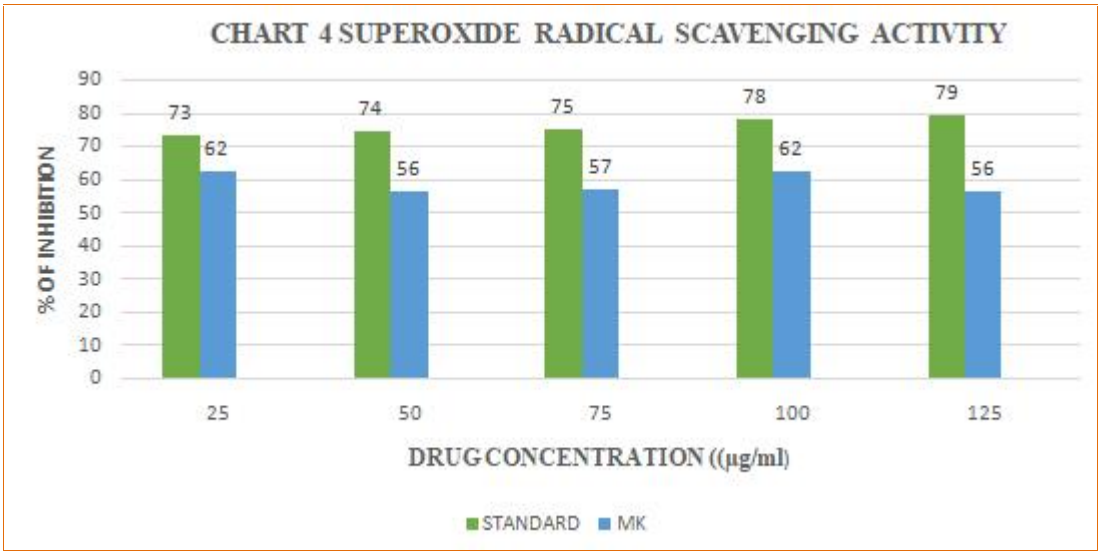


*** MK - Malattu Karpam**

Superoxide radical scavenging activity by alkaline DSMO method

Table 6

S.No	Drug Conc (µg/ml)	Std	% of inhibition	Test 1	% of inhibition
1	25	0.857	73%	1.204	62
2	50	0.816	74%	1.406	56
3	75	0.782	75%	1.37	57
4	100	0.692	78%	1.204	62
5	125	0.667	79%	1.406	56



*** MK - Malattu Karpam**

Discussion

From the text Theraiyar Yemaha Venba, the trial drug Malattu Karpam was chosen to test the medicine's antioxidant properties (Invitro Assay).

The medication was made in accordance with the protocol and put through a number of tests to determine its efficacy and potency against the illness. A literature research of the components of Malattu Karpam from different textbooks offered optimism on its efficacy.

Literary review, which consists Botanical Aspect, Gunapadam Aspect and Pharmacological review which support this study.

DPPH Radical scavenging assay

In the present study, the extract of Malattu Karpam was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the Malattu Karpam extract was given in (Table 1). The extract of Malattu Karpam showed the highest DPPH scavenging activity (77%) at 40µg/ml and the lowest percentage of inhibition (50%) at 10µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (85%) at 50µg/ml and the lowest percentage of inhibition (72%) at 10µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and Malattu Karpam extract. The Malattu Karpam extract has more or less equal DPPH scavenging activity when compared to the standard. From the present study, it was concluded that the Malattu Karpam extract has a marked antioxidant activity at higher concentrations.

Nitric oxide radical scavenging activity

The extract of Malattu Karpam was found to possess concentration dependent scavenging activity on nitric oxide radicals. The values of NITRIC OXIDE radical scavenging activity of the Malattu karpam extract was given in (Table 2). The extract of Malattu Karpam showed the highest NITRIC OXIDE scavenging activity (68%) at 50µg/ml and the lowest percentage of inhibition (62%) at 10µg/ml. DMSO (Standard) showed highest percentage of inhibition (72%) at 50µg/ml and the lowest percentage of inhibition (57%) at 10µg/ml.

This indicated that % of inhibition increased with increase in concentration of both the standard and Malattu Karpam extract. The Malattu Karpam extract has more or less equal NITRIC OXIDE scavenging activity when compared to the standard. From the present study, it was concluded that the Malattu Karpam extract has a marked antioxidant activity at higher concentrations.

Total iron reducing power assay

The extract of Malattu karpam was found to possess concentration dependent total iron reducing power assay. The values of total iron

reducing power assay of the Malattu karpam extract was given in (Table 3). The extract of Malattu Karpam showed the highest total iron reducing power assay (88%) at 50µg/ml and the lowest percentage of inhibition (64%) at 10µg/ml. Standard showed highest percentage of inhibition (78%) at 50µg/ml and the lowest percentage of inhibition (50%) at 10µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and Malattu Karpam extract. The Malattu Karpam extract has more or less equal total iron reducing power assay when compared to the standard. From the present study, it was concluded that the Malattu Karpam extract has a marked antioxidant activity at higher concentrations.

Superoxide radical scavenging activity by alkaline DMSO method

The extract of Malattu karpam was found to possess concentration dependent scavenging activity on nitric oxide radicals. The values of Superoxide radical scavenging activity of the Malattu Karpam extract was given in (Table 4). The extract of Malattu Karpam showed the highest superoxide scavenging activity (62%) at 100µg/ml and the lowest percentage of inhibition (56%) at 50µg/ml. DMSO (Standard) showed highest percentage of inhibition (79%) at 125µg/ml and the lowest percentage of inhibition (73%) at 25µg/ml. This indicated that % of inhibition increased with increase in concentration of both the standard and Malattu Karpam extract. The Malattu Karpam extract has more or less equal superoxide scavenging activity when compared to the standard. From the present study, it was concluded that the Malattu Karpam extract has a marked antioxidant activity at lowest concentrations.

Antioxidant activity of trial drug Malattu Karpam was found to be 50% to77 %, 62% to 68%, 64% to 88% for DPPH assay, Nitrous oxide assay ,Total iron reducing power assay respectively When compared to Standard drug Ascorbic acid and gallic acid they had more Antioxidant activity i.e., 72% to 85%, 57% to 72% and 50% to 78% respectively. Thus, trial drug had high antioxidant activity when compared to standard synthetic compound but it sure the trial drug also had antioxidant activity.

Thus, the drug had high antioxidant activity when compared to standard drug. Standard drug is single compound while trial drug is mixture of raw drugs. And dosage of the trial drug is approximately 5 – 10gm but here 10mg – 50mg of trial drug is tested but these amounts also had enough amount of antioxidant activity.

Conclusion

The In-vitro anti-oxidant assay results for the Malattu Karpam sample led to the conclusion that the siddha formulation of Malattu Karpam exhibits promising anti-oxidant activity in the estimated DPPH, Nitrous oxide, Total iron reducing assay and Super oxide radical scavenging assay. Antioxidant substances have the same activity as Karpa Marunthu as indicated in the original Siddha text they inhibit ageing and cell death. According to scientific criteria, the above results for Siddha text are accurate.

To assess their efficacy and provide solid support for Siddhar's science, preclinical and clinical research will be conducted in the future.

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