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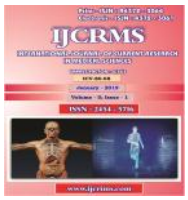

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In-vitro study of Free Radicle Scavenging activity of Poly Herbal Siddha formulation "*Siringipaerathi Chooranam*" by DPPH assay.

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Abstract

Traditional system of medicine have been in trend for treating various diseases in many developing and developed countries. However this system plays a major role in preventive and curative in many diseases due to the presence of their phyto constituents. The supernatural scientists were the Siddhars who gave a boon to reduce aging process. The aim of the current research is to explore the poly herbal formulation "*Siringipaerathi Chooranam*" the Antioxidant property by DPPH radical scavenging assay in *in-vitro* study. The antioxidant activity of test drug sample SPC was determined by using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay at the concentration of 1.25 µg/ml, 2.5 µg/ml,5 µg/ml,10 µg/ml and 20 µg/ml using ascorbic acid as standard. Results of the DPPH free radical scavenging assay of *SPC* shows the highest percentage inhibition of about at 73.91% at 20µg/ml when compare to standard ascorbic acid with 89.62%. In conclusion the trial drug *SPC* possess significant antioxidant property which contributes to the beneficial effect of oxidative stress with associated disorders.

Keywords: Siddha medicine, DPPH, Antioxidant, Herbs, *Siringipaerathi Chooranam*, *SPC*.

Introduction

Siddha system of medicine is one of the traditional system of medicine, which is considered to be the art of healing and provided the alignment for humans through the medications. Siddha system of medicine is unique because of its versatile medical preparations. However most of the Siddha formulations were herbal preparations, Thus the benefits of herb and extracts have been discussed for centuries[1]

This system of medicine comprises of Kaya karpam, which was the major components of Anti oxidants. Kaya karpam was classified into two types 1. General which is meant for prophylaxis, prevention, ageing, shrinking of skin etc. 2. Special which is meant for therapeutics for ex: long term use of pepper for tenial infections. General karpam was used at any time but Special karapam was given for specific diseases[2].

Kaya karpam which has the major components of Anti-oxidants are classified into two types namely General (meant for prophylaxis, for the prevention of ageing, whitening of hairs, shrinking of skin etc. Example is Honey ginger, *Aloe vera* etc) and Special (meant for Therapeutics for example, Long pepper was taken internally for a month, cures Tenial infections).

The General karpam can be taken at any time and the special is to be taken for treating specific diseases.

Anioxidant are the substances that inhibits oxidation of the molecules in the way of terminating initiation or propagation in oxidizing chain reaction. Free radicals are the atoms with unpaired number of electrons formed when oxygen interacts with certain molecules. Once formed were highly reactive radicals can start in a chain reaction, Free radicals were harmful to the body and damage the components of cells, DNA, proteins, and cell membranes[3]

Oxidative stress was identified as the cause of development several diseases. The exogenous antioxidants or endogenous antioxidant defenses the body with the undesirable effects of reactive oxygen species (ROS) induced oxidative damage. Herbs plays a wide role in the attenuating ROS induced oxidative damage. Antioxidants significantly play a major role in delay or prevents the oxidation of oxidizable substrates when present at lower concentrations than the substrate[4]

There were several herbals has been validated for antioxidant property in *in-vitro* models. In order to explore the Siddha medicine to the World I have prepared to choose *SPC* is a poly herbal formulation of Siddha medicine which has the potential of reducing ROS.

Materials and Methods

Drug selection:

In this research paper purified and prepared *"Siringipaerathi Chooranam"* was taken as a trial drug for Hepatoprotective activity from the Siddha literature *"Sarabendra Vaidhiya Muraigal". Soolai, Moola, Kusta, Pitharoga Muraigal*, page no: 194-195.

Table: 1. Ingredients:

Name of drugs	Botanical name	Quantity
Inji	*Zingiber officinalis*	560 gm (16 palam)
Milagu	*Piper nigrum*	50.4gm (12 varahan)
Thippili	*Piper longum*	33.6gm(8 varahan)
Thipili moolam	*Piper longum*	16.8gm(4 varahan)
Lavanga pathiri	*Cinnamomum tamala*	35gm(1 palam)
Elam	*Elettaria cardamomum*	42gm (10 varahan)
Kodiveli ver	*Plumbago zeylanica*	42gm (10 varahan)
Lavanga pattai	*Cinnamomum zeylanicum*	35gm (1 palam)
Moongil uppu	*Bambusa arundinaceae*	35gm (1 palam)
Sandhana thool	*Santalum album*	35gm (1 palam)
Vilamichu-ver	*Plectranthus vettiveroides*	35gm (1 palam)
Sathikkai	*Myristica fragrans*	35gm (1 palam)
Seeragam	*Cuminum cyminum*	35gm (1 palam)
Kirambu	*Syzygium aromaticum*	35gm (1 palam)
Sugar	*Saccharum officinarum*	Equal quantity
Nei	English Name : Ghee	30ml

Collection of the Plant materials:

All the raw materials were bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug:

All the plant materials were identified and authenticated by the Botanists and Gunapadam experts in Government Siddha Medical College, Arumbakkam, and Chennai-106.

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature [5].

Inji	Outer skin of ginger was peeled off.
Milagu	It was soaked in sour buttermilk for 3 hours and allowed to dry
Thippili	Soaked in lemon juice and allowed to dry.
Thippilimoolam	Remove the nodules and dried.
Lavangapathiri	Dried in sun light.
Elam	Roasted in the pan and outer skin was removed.
Kodiveli-ver	The root was cleaned with a white cloth.
Lavangapattai	Dried in sun light.
Sandhana kattai	The skin was peeled off to get purified and powdered
Vilamichu-ver	The root was cleaned with a white cloth.
Sathikkai	Cleaned and cut into small pieces and dried.
Seeragam	Clean the dust particles and allowed it to dry.
Kirambu	Flower buds were removed.

Preparation of the Drug:

Procedure:

In order to obtain the purified form of ginger, the upper skin of ginger was peeled off and then sliced into small pieces. The sliced pieces were dried in sunshade for two days. After complete drying 560 grams of dried ginger was taken and fried well in ghee and then powered.

50.4 grams of Purified *Pepper*, 33.6 grams of *Thippili*, 16.8 grams of *Thipplimoolam*, 42 grams of *Kodiveli-ver*, 35 grams of *Moongil uppu*, *Lavangapathiri*, *Sandhana thool*, *Vilamichu-ver*, *Lavanga Pattai*, *Adhikari*, *Seeragam*, *Kirambu* were taken and powered separately then mixed together with processed ginger powder. Finally, the mixture was ground well which favors the homogenous preparation. Then the mixture powder was sieved through the thin clean white cloth. After that twice the weight of sugar was added to the mixture and again it was ground well. Finally, the end product was obtained, which was kept in an air tight container and labeled as "***Siringipaerathi Chooranam" (SPC)*** [6].

Purification of the Chooranam: steaming process (*Pittaviyal murai*)

The *"Siringipaerathi Chooranam"* was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. A mud pot was taken and it was half filled by milk and mixed with equal quantity of pure water. The mouth of the pot was sealed by a cloth. This *Chooranam* was placed over a clean dry cloth and tied firmly around the mouth of mud pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk reduced in the lower pot. The same drug was later dried and powdered then sieved again. It was used for the further study [7].

Storage of the drug:

The prepared test drug was stored in a clean, air tight glass container.

Administration of the drug:

Form of the medicine : *Chooranam*
Route of Administration : Enteral
Dose : 2 gm twice a day depending on the severity
Adjuvant : honey

Indication:

Kamaalai, *Marbuvali, Kirani, Suram, Vaanthi, Peenisam*.

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay:

The antioxidant activity property of test drug *SPC* was determined by 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . The test drug sample *SPC* was mixed with 95% methanol for prepare the stock solution in a required concentration. From the stock solution the concentration of the serial dilution was made with 1.25µg/ml, 2.5 µg/ml,5 µg/ml,10 µg/ml and 20 µg/ml. Ascorbic acid was used as a standard, which was prepared in the same concentrations as that of the sample extract by using methanol as solvent. Finally the reaction mixture, which containing 1 ml of 0.3 mm of DPPH methanol solution was added to the 2.5 ml of the sample solution in different concentrations and allowed to react at 370C room temperature. Absorbance in the presence of test sample *SPC* at different concentration of (1.25µg, 2.5µg, 5 µg, 10 µg and 20µg/ml) were noted after 15 min incubation period at 370C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

The effective concentration of the test sample SPC required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentration[8].

Results and Discussion

Anti-oxidant activity:

Table: 2.DPPH Assay of *Siringipaerathi Chooranam*

Sample concentration (µg/ml)	Percentage of Inhibition	
	SPC	**Standard**
Control	-	-
1.25	19.14248	40.89
2.50	29.7287	51.25
5	56.80137	74.07
10	65.18687	83.33
20	73.91387	89.62

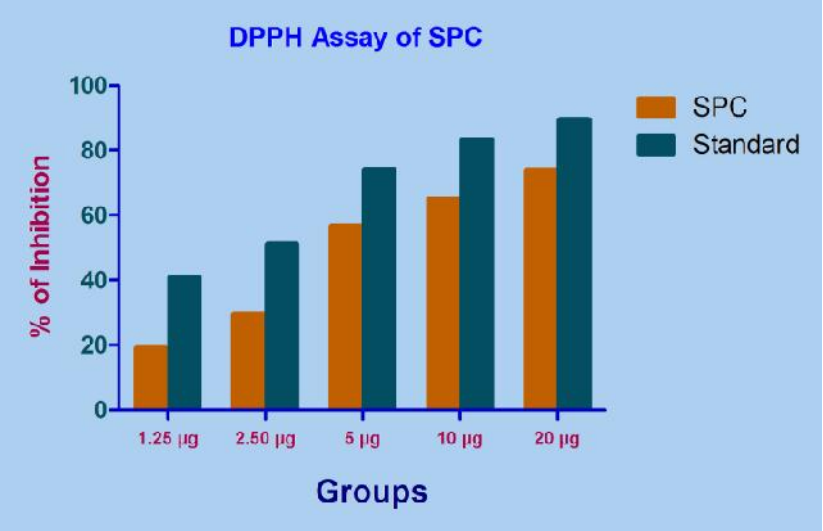

Chart: 1.DPPH Assay of *Siringipaerathi Chooranam*

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *SPC* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colourless stable molecule1,1diphenyl-2-picryl hydrazil is formed and a suit of which the absorbance at 517 nm of the solution is decreased. In the present study, the *SPC* extract was analyzed was able to decolourize DPPH. In the present study, the extract of *SPC* was found to possess concentration dependent scavenging activity on DPPH radicals. The values of DPPH free radical scavenging activity of the *SPC* extract was given in Table. The extract of *SPC* showed the highest DPPH scavenging activity (73.91%) at 20µg/ml .Ascorbic acid (Standard) showed highest percentage of inhibition (89.62%) at 20µg/ml.

This indicated that % of inhibition increased with increase in concentration of both the standard and *SPC* extract. The *SPC* extract has more or less equal DPPH scavenging [9].

Conclusion

The oxidative stress leads to many damages to the nucleic acids, proteins, lipids and also cause various diseases. In Order to overcome the effects an attempt was made in in-vitro study. In this paper the author explore the antioxidant property of *SPC* by DPPH Assay.

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