ISSN: 2322- 0902 (P)
ISSN: 2322- 0910 (O)

International Journal of Ayurveda and Pharma Research

Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF *SEERAGA CHOORANAM* IN- VITRO ASSAY (A SIDDHA POLYHERBAL PREPARATION)

R Keerthana[1]*, S Visweswaran[2], S Sivakkumar[3], A. Maariappan[3], V.Banumathi [4]

*[1]PG Scholar, [2]HOD I/C, [3]Lecturer, Department of Gunapadam, National institute of Siddha, Tambaram Sanatoruim, Chennai, Tamil Nadu.

[4]Director, National Institute of Siddha Chennai, Tambaram Sanatoruim, Chennai, Tamil Nadu.

ABSTRACT

Siddha Medicine (Tamil Citta- or Tamil-maruttuvam) is a system of traditional medicine originating in Tamil Nadu in South India. The drugs used by the Siddhars could be classified into three groups: *Thaavaram*, *Thaathu* and *Jangamam seeraga chooranam* is a drug under the category of *Thaavaram*. *Seeraga choornam* is a poly herbal formulation which is mentioned in the text Pullipanivaithyam 500 which has been therapeutically used for *Pithanoigal, Kirukirupu, Vaanthi, Mayakkam, Ajeeranam, Kaangai, Veppam.* Siddha system emphasis mainly on healthy long life by preventing ageing and degenerative disease with medicines having antioxidant activity. Even though this drug has been used based on traditional knowledge no scientific work has been done to evaluate the antioxidant properties of *Seeraga choornam* (SC) based on various in vitro assays. The study result confirmed that the drug *Seeraga choornam* has promising therapeutic antioxidant activity when compared with the standard drug. This research work can help for medical practitioners to use this polyherbal compound for the treatment of cancer.

KEYWORDS: *Seeraga Chooranam*, Antioxidantactivity, Polyhrebal, DPPH.

INTRODUCTION

A Siddha system of medicine is the oldest, traditional and holistic system with which is being practiced by a large population in south India.[1] The development of this traditional system of medicines with perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in health care.[2] Herbal drugs have found wide spread use in many countries not only because they are easily available and are cheaper but an important reason has been the notion that they are safer than synthetic drugs which may not always be true[3] not only in form of drugs even in cooking, many indigenous plants are used as the major ingredient[4] Siddha medicine (*Chooranam*), a mixture of powdered herbs which helps in body regulation and nowadays the same is formulated into tablets in order to fix the dose for easy intake which makes it more convenient for the people. *Seeraga choornam* (SC) is a poly herbal formulation which has been therapeutically used for *Pithanoigal, Kirukirupu, Vaanthi, Mayakkam, Ajeeranam, Kaangai, Veppam.*[5] *Seeraga chooranam*, composed of multiple herbs such as *Cuminum cyminum, Emblica officinalis, Melia azadirachta* as mentioned in the table 1. The cumin plant (*Cuminum cyminum*), the main ingredient helps to get relieved from indigestion problem. *Cuminum cyminum* L. (Cumin) belongs to the family Apiaceae. The antioxidant property of cumin was reported earlier.[6] It is used as anti-parasitic and antimicrobial agent which reduces fever and also serves to be a good pain killer. The antibacterial activity of cumin on Klebsiella pneumonia was studied.[7] Major components in the cumin oil are 1,8-cineole, αterpineol, DL-limonene, nerolidol, 4-terpineol,δ-terpineol and longifolenaldehyde.[8] In addition, this component has a multiple role in the treatment of a variety of problems. Similar to this even the other compounds present helps to recover from many medical problems. The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward functional food with specific health effects. [9] So this study was performed to investigate the Anti-oxidant activity in *Seeraga choornam* based on various in vitro assay.

AIM AND OBJECTIVE

The aim of this study is to evaluate the in vitro Anti oxidant activity for the drug *Seeraga Chooranam*.

MATERIALS AND METHODS

Sample Collection

The sample was collected from IMPCOPS pharmacy in Thiruvanmiyur, Chennai.

Ingredients of the Drug : *Seeraga chooranam* are given below.

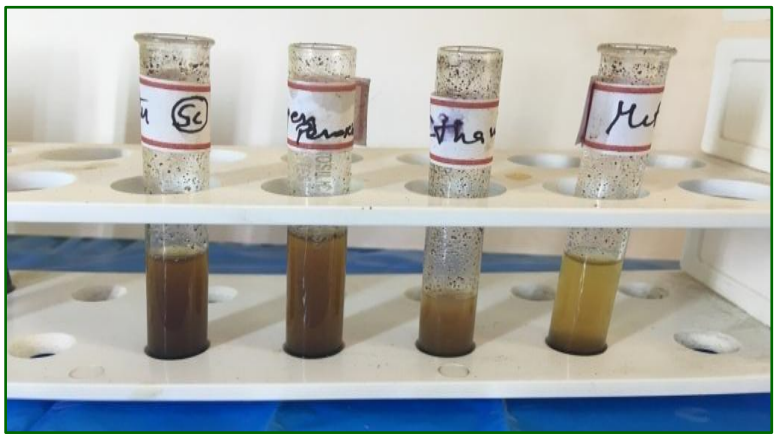
Table 1: *Seeraga choornam*

S.No.	Siddha Name	Botanical Name
1	*Seerakam*	*Cuminum cyminum*
2	*Sarkarai*	Sucrose
3	*Elumichampazhasaaru*	*Citrus limon*
4	*Karumbusaaru*	*Saccharum officinarum*
5	*Musumusukkaisaaru*	*Mukia scabrella*
6	*Nellikaisaaru*	*Emblica officinalis*
7	*Thulhuvalaisaaru*	*Solanum trilobatum*
8	*Thumbaielaisaaru*	*Leucas aspera*

Sample Description

State	Solid
Appearance	Brownish Black
Nature	Moistured Soft Fine powder
Odor	Strong Characteristic

Solubility Assay



S.No.	Solvent Used	Solubility
1.	Water	Highly Soluble
2.	Methanol	Soluble
3.	Ethanol	Soluble
4.	Hydrogen Peroxide	Soluble

Anti Oxidant Properties

DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay[10]

The antioxidant activity of test drug sample SC was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample SC was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution 1ml, 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10ml whose concentration was then10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the test drug by using methanol as solvent. Final reaction mixture containing 1ml of 0.3mm DPPH methanol solution was added to 2.5ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample SC at different concentration of (10µg, 20µg, 40µg, 60µg, 80µg and 100µg/ml) was noted after 15 min incubation period at 37^{0}C. Absorbance was read out at 517nm 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 test sample SC required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between percentage inhibition and concentrations.

Nitric Oxide Radical Scavenging Assay[11]

The concentrations of test sample SC are made into serial dilution from 10−100µg/ml and the standard gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5ml of 10mm sodium nitroprusside in phosphate buffered saline was mixed with 1ml of the different concentrations of the test drug (10−100 µg/ml) and incubated at 25°C for 180 mins. The test drug SC was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm using a Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug SC and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug SC and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged } (\%) = \frac{A_{control} - A_{test}}{A_{control}} \times 100,$$

where $A_{control}$ = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

ABTS Assay[12]

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug SC against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals. The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5ml of 7mm ABTS with 8µL of 140mm potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1:44, v/v). To determine the scavenging activity, 100µL ABTS reagent was mixed with 100µL of test sample (10-100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug SC was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample SC was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

$$\text{Radical scavenging } (\%) = \left[\frac{(A)_{control} - (A)_{sample}}{(A)_{control}}\right] \times 100.$$

Hydrogen Peroxide Radical Scavenging Assay

A hydrogen peroxide solution (2mm) was prepared in 50mm phosphate buffer (pH 7.4). Aliquots (0.1ml) of the test sample SC (different concentration ranging from 10-100µg/ml) were transferred into the test tubes and their volumes were made up to 0.4ml with 50mm phosphate buffer (pH 7.4). After adding 0.6ml of hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230nm was determined after 10min, against a blank. BHA was used as the positive control. The percentage inhibition of the test drug SC and standard was calculated and recorded. The percentage radical scavenging activity of the test drug SC and BHA were calculated using the following formula:

$$\text{Radical scavenging } (\%) = \left[\frac{(A)_{control} - (A)_{sample}}{(A)_{control}}\right] \times 100$$

RESULTS

Percentage inhibition of test drug SC on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of SC	% Inhibition of Ascorbic Acid
10 µg/ml	4.474 ± 1.82	45.06 ± 2.12
20 µg/ml	16.4 ± 2.19	56.41 ± 3.73
40 µg/ml	29.39 ± 3.34	64.57 ± 7.08
60 µg/ml	38.86 ± 0.67	72.37 ± 5.45
80 µg/ml	45.88 ± 1.68	79.11 ± 2.12
100 µg/ml	62.37 ± 1.53	93.61 ± 0.22

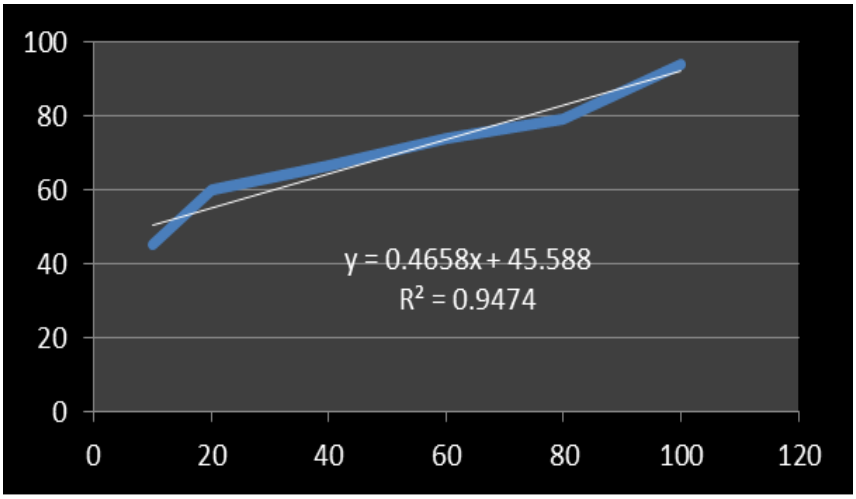
Data are given as Mean ± SD (n=3)

IC50 Values for DPPH radical scavenging Assay by SC and standard

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
Ascorbic Acid	13.46 ± 5.543
SC	88.37 ± 13.81

Data are given as Mean ± SD (n=3)

Percentage inhibition of STD on DPPH radical scavenging assay



Percentage inhibition of test drug SC on Nitric Oxide radical scavenging assay

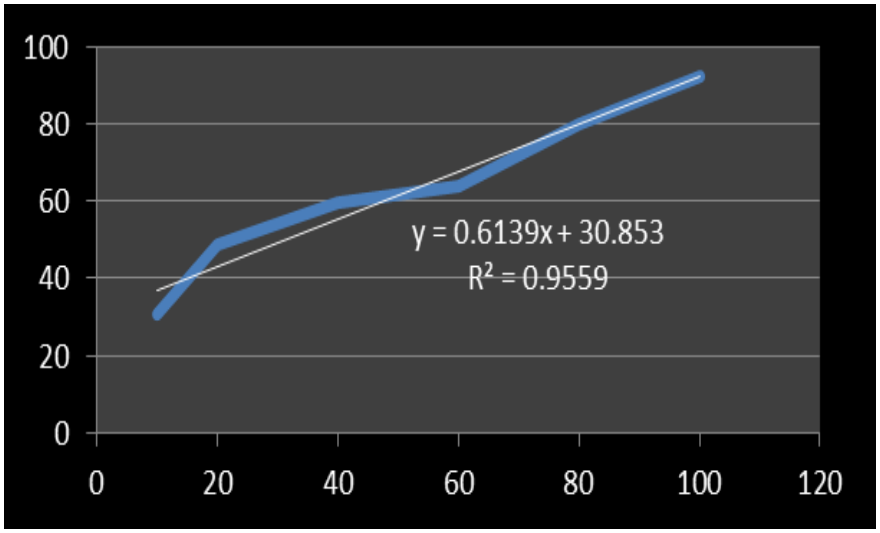
Concentration (µg/ml)	% Inhibition of SC	% Inhibition of Gallic Acid
10µg/ml	8.582 ± 3.962	33.14 ± 4.346
20µg/ml	15.91 ± 3.859	47.84 ± 2.845
40µg/ml	25.43 ± 6.249	59.67 ± 1.075
60µg/ml	32.03 ± 6.249	65.76 ± 4.071
80µg/ml	36.9 ± 8.911	79.02 ± 2.845
100µg/ml	48.88 ± 5.639	92.25 ± 0.1075

Data are given as Mean ± SD (n=3)

IC50 Values for Nitric Oxide radical scavenging assay by SC and standard

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
SC	106.4 ± 19.53
Gallic Acid	29.99 ± 4.946

Data are given as Mean ± SD (n=3)



Percentage inhibition of test drug SC on ABTS radical scavenging assay

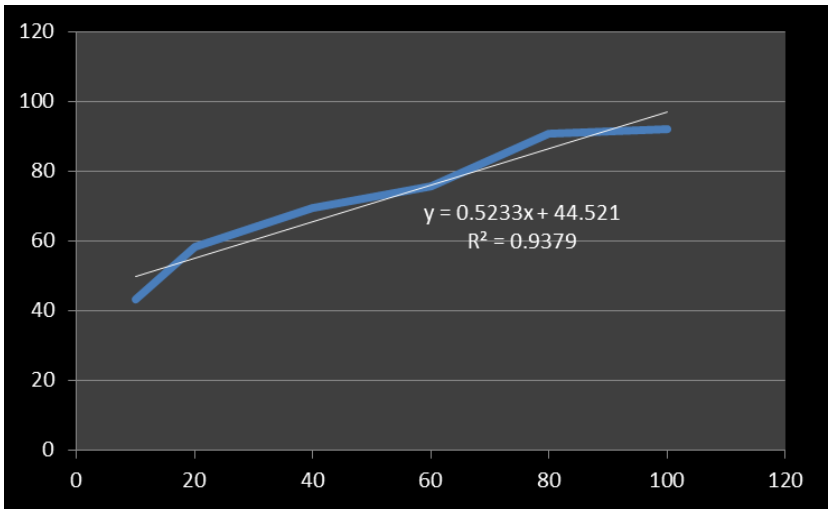
Concentration (µg/ml)	% Inhibition of SC	% Inhibition of Gallic Acid
10µg/ml	7.632 ± 2.78	43.96 ± 2.26
2µg/ml	15.7 ± 1.21	56.63 ± 2.6
40µg/ml	24.47 ± 2.75	67.13 ± 2.83
60µg/ml	38.86 ± 4.32	75.45 ± 2.73
80µg/ml	46.23 ± 4.24	84.64 ± 5.36
100µg/ml	57.81 ± 6.18	91.9 ± 0.11

Data are given as Mean ± SD (n=3)

IC50 Values for ABTS radical scavenging assay by SC and standard

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
SC	85.61 ± 9.861
Gallic Acid	13.9 ± 8.279

Data are given as Mean ± SD (n=3)



Percentage inhibition of test drug SC on Hydrogen peroxide radical scavenging assay

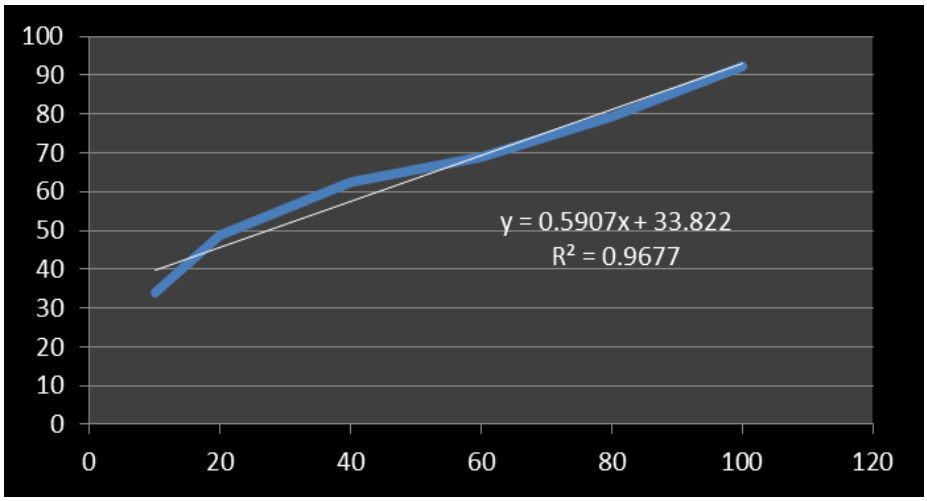
Concentration (µg/ml)	% Inhibition of SC	% Inhibition of BHA
10µg/ml	4.051 ± 0.6046	36.57 ± 2.189
20µg/ml	10.68 ± 1.047	48.83 ± 2.103
40µg/ml	17.32 ± 1.6	60.4 ± 2.782
60µg/ml	22.2 ± 1.047	69.16 ± 2.646
80µg/ml	27.09 ± 2.635	77.22 ± 2.103
100µg/ml	33.72 ± 3.775	93.35 ± 1.428

Data are given as Mean ± SD (n=3)

IC50 Values for Hydrogen peroxide radical scavenging assay by SC and standard

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
SC	153.1 ± 16.72
BHA	26.9 ± 1.148

Data are given as Mean ± SD (n=3)



DISCUSSION

Seeraga chooranam (SC) was screened for DPPH radical scavenging activity and the percentage inhibition ranges from 4.47% to 62.37% when compared with standard ascorbic acid with percentage inhibition ranges from 45.06% to 93.61%. The IC50 value of the trial drug was found to be 88.37(µg/ml) when compared with standard ascorbic acid with (IC_{50}value 13.46µg/ml). No radical scavenging activity of the *Seeraga chooranam* revealed that the percentage inhibition of the test drug ranges from 8.58% to 48.88% when compared with standard Gallic acid with percentage inhibition ranges from 33.14% to 92.25%. The corresponding IC50 value of the *Seeraga chooranam* was found to be 106.4 (µg/ml) when compared with standard gallic acid with (IC_{50} value 29.99µg/ml). *Seeraga chooranam* were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 7.63% to 57.81% when compared with standard gallic acid with percentage inhibition ranges from 43.96% to 91.9%. The corresponding IC50 value of the *Seeraga chooranam* was found to be 85.61(µg/ml) when compared with standard gallic acid with (IC_{50}value 13.9µg/ml) *Seeraga chooranam* were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 4.05% to 33.72% when compared with standard BHA with percentage inhibition ranges from 36.57% to 93.35%. The corresponding IC50 value of the *Seeraga chooranam* was found to be 153.1 (µg /ml) when compared with standard BHA with (IC_{50}value 26.9µg/ml).

CONCLUSION

From the findings of the present study, the antioxidant properties of *Seeraga choornam* were revealed scientifically. The antioxidant property of Siddha formulation, *Seeraga choornam* was studied based on various in vitro assays for the first time.

Seeraga chooranam has promising anti-oxidant activity and provides scientific support for employing this polyherbal drug for therapeutic use in Indian system of medicine.

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Cite this article as:
R Keerthana, S Visweswaran, S Sivakkumar, A. Maariappan, V.Banumathi. Evaluation of Antioxidant activity of seeraga Choornam in- vitro assay (A Siddha polyherbal preparation). International Journal of Ayurveda and Pharma Research. 2018;6(7):17-23.

Source of support: Nil, Conflict of interest: None Declared

***Address for correspondence**
Dr. R Keerthana
PG Scholar,
Department of Gunapadam,
National institute of Siddha,
Tambaram Sanatoruim, Chennai,
Tamil Nadu, India.
Email: sugeebsms@gmail.com