Original Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF *PARANGIPPATTAI RASAYANAM* - A SIDDHA POLYHERBAL FORMULATION

ABSTRACT:

Background: The Siddha system of medicine uses a fascinating combination of herbs, minerals and metals to promote good health and longevity. *Parangippattai Rasayanam* is a polyherbal formulation mentioned in the Siddha literature and is indicated for *Soolai* (Pain), *Viranam* (Various ulcers), *Kiranthi* (Venereal diseases), *Kuttam* **(**Skin diseases), *Gunmam* (peptic ulcer) and *Moorchai* [1].

Aim: To evaluate the in-vitro antioxidant activity of Parangipattai Rasayanam

Materials and methods: The antioxidant activity of *Parangippattai Rasayanam* was evaluated by using DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay, Nitric Oxide Radical Scavenging Assay, ABTS Assay, Hydrogen Peroxide Radical Scavenging Assay.

Result: *Parangippattai Rasayanam* has promising anti-oxidant activity in the estimated assays.

Comment [u1]: How you can present it in statistical terms.

KEYWORDS: Siddha medicine, Polyherbal formulation, *Parangipattaai Rasayanam*, *Kuttam*, Anti-oxidant properties.

INTRODUCTION:

Siddha system of medicine is one of the oldest medical systems of India that existed separately in early times. The system has flourished well in India for many centuries. Although this system has declined in later years, in the wake of changing mode of life and modern medicine, it continues to sustain its influence on the masses because of its incomparable intrinsic merits. Siddha medicine can combat all types of diseases, especially chronic diseases, which baffles and eludes even modern sophisticated medicine.

Kayakarpam is one of the unique special therapeutic divisions in the Siddha system of medicine advocated especially for rejuvenation, decreasing morbidity, and increasing the life span. “Kayam” means body and “Karpam” means ‘strong as stone’. Hence it means keeping the body as strong as stone [2]. Kayakarpam provides both mental and physical wellness to the individual. In recent years lifestyle modification is one of the main causes of many health problems including many non-communicable diseases. Kayakalpa herbs are rich in natural sources of antioxidants and so it necessitates turning towards such medicines to meet this great threat.

In recent years, there has been a great deal of attention toward the field of free radical chemistry. Free radicals reactive oxygen species and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states. Production of free radicals results in oxidative stress due to damage of DNA, proteins, lipids and has been suggested to be the cause of most serious human diseases [3]. 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. Free radicals thus adversely alter lipids, proteins, DNA and trigger a number of human diseases. Hence the researchers are searching for a potent antioxidant drug

from natural resources. In ancient times, this has been clearly mentioned in the Siddha system of medicine as Kaaya Karpam Therapy (Rejuvenation).

Natural antioxidants are considered to be safe and bioactive [4]. The antioxidants from natural sources are the only alternative to synthetic antioxidants in counteracting the free radicals associated with disease [5]. Phytosterols, Flavonoids,Amino acids, Terpenoids, Phenolic Compounds and Tannins, Saponins, Carbohydrates were present in Parangipattai Rasayanam. The antioxidant activities of phenolic compounds are mainly due to the redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers, in addition to their metal-chelating potential. The antioxidant activity of phenolics plays an important role in the adsorption or neutralization of free radicals [6]. In recent years, various species of plants have been used in the preparation of drugs and are consumed as food due to their antioxidant activities [7]. Therefore, antioxidants with free radical scavenging activities of medicinal plants may have great relevance in the prevention of diseases and in therapeutic properties [8]. Plants, rich in their phytochemical compounds, are good sources of antioxidants and radical scavengers [9].

The traditional Siddha medicine has a holistic approach to life, equilibrium of the mind and body with the environment and an emphasis on health rather than on disease [10]. *Parangipattai Rasayanam (*PRM) is a classic Siddha drug chosen from the text *Pulippani vaithiyam-500.* It is indicated for *Soolai* (Pain), *Viranam* (Various ulcers), *Kiranthi* (Venereal diseases), *Kuttam* **(**Skin diseases), *Gunmam* (peptic ulcer) and *Moorchai*. The main objective of the study was to determine the antioxidant activity of PRM by DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay, Nitric Oxide Radical Scavenging Assay, ABTS Assay, Hydrogen Peroxide Radical Scavenging Assay.

MATERIALS AND METHODS:

Standard Operating Procedure of *Parangippattai Rasayanam*:

Collection of Raw Drugs:

The required raw drugs for the preparation of '*Parangipattai Rasayanam'* were procured from the Country Medicine shop, Parrys, Chennai and from Kanyakumari.

Raw drugs Identification and authentication:

These ingredients were identified and were authenticated by Medicinal Botanist at NIS, Tambaram Sanatorium, Chennai.

Purification processes of ingredients of PRM:

The ingredients were purified as per the methods stated in the Siddha literature. The raw drugs were purified in the Gunapadam Laboratory of the National Institute of Siddha.

Ingredients:

Sangam ver (Azima tetracantha Linn)	: 35grams.
Peesangam ver (Clerodendrum inerme Linn)	: 35grams.
Chithiramoola ver (Plumbago zeylanica,Linn)	: 35grams.
Nilappanai kizhangu (Curculigo orchioides)	: 35grams.
Amukkara kizhangu (Withania somnifera.Dunal,)	: 35 grams.
Kumilam ver (Gmelina arborea)	: 35 grams.
Nilakkumilam ver (Gmelina asiatica)	: 35 grams.
Nerunjil ver (Tribulus terrestris)	: 35 grams.

Poovarasam pattai (Thespesia populnea)	: 35 grams.
Sengaththari pattai (Capparis sepiaria)	: 17.5grams.
Chukku (Zingiber officinale.Roscoe.)	: 17.5grams.
Thippili (Piper longum.Linn.)	: 17.5 grams.
Milagu (Piper nigrum.Linn.)	: 17.5 grams.
Omam (Carum copticum)	: 17.5 grams.
Sirulavanga pattai (Cinnamomum verum)	: 17.5grams.
Kostam (Costus speciosus)	: 17.5grams.
Sirunaagap poo (Mesua nagassarium)	: 17.5grams.
Citarathai (Alpinia galangal)	: 17.5grams.
Inji (Zingiber officinale)	: 17.5grams.
Lavanga illai (Syzygium aromaticum)	: 17.5grams.
Parangi chakkai (Smilax china Linn.)	: 175 grams.
Sugar	: 350 grams.
Honey	: 700 grams.
Ghee	: 700 grams.

Preparation:

All the above raw drugs were made into a fine powder. This powder was then mixed with sugar, honey, and ghee to the consistency of *Rasayanam.*

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

The antioxidant activity of test drug sample PRM was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample PPR was mixed with 95% methanol to prepare the stock solution in the 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 the same solvent was made the final volume of each test tube up to 10 ml whose concentration was then10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Ascorbic acid was used as a standard and was prepared in the same concentration as that of the test drug by using methanol as solvent. The final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample PPR at different concentrations of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/ml) was noted after 15 min incubation period at 37^0C. Absorbance was read out at 517 nm using a 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 PRM required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations [11].

Nitric Oxide Radical Scavenging Assay

The concentrations of test sample PRM are made into serial dilution from 10−100 µg/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% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10−100 µg/mL) and incubated at 25°C for 180 mins. The test drug PRM 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 PRM and standard was calculated and recorded [12]. The percentage nitrite radical scavenging activity of the test drug PRM and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

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

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

ABTS Assay

This assay was carried out for the purpose of evaluating the anti-oxidant potential of test drug PRM 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 5 mL of 7 mM ABTS with 88 µL of 140 mM 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, 100 µL ABTS reagent was mixed with 100 µL of the test sample (10-100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured at 734 nm. 100% methanol was used as a control. Gallic acid with the same concentrations of test drug PRM was measured following the same procedures described above and was used as positive controls [13]. The antioxidant activity of the test sample PRM was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

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

Hydrogen Peroxide Radical Scavenging Assay

A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample PRM (different concentrations ranging from 10-100µg/ml) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. BHA was used as the positive control [14]. The percentage inhibition of the test drug PRM and standard was calculated and recorded. The percentage radical

scavenging activity of the test drug PRM and BHA were calculated using the following formula:

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

RESULTS AND DISCUSSION

Comment [u2]: How you can present it in statistical terms overall

Comment [u3]: Include a paragraph of discussion of how you explain from your view the objective stated.

DPPH radical scavenging activity

Table:1 Percentage inhibition of test drug PRM on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of PRM	% Inhibition of Ascorbic Acid
10 µg/ml	5.912 ± 1.771	30.4 ± 4.861
20 µg/ml	16.82 ± 5.513	42.71 ± 2.525
40 µg/ml	22.63 ± 8.056	63.21 ± 1.311
60 µg/ml	35.36 ± 3.708	71.7 ± 4.69
80 µg/ml	46.26 ± 2.077	82.63 ± 4.74
100 µg/ml	54.98 ± 4.249	97.38 ± 1.595

Data are given as Mean ± SD (n=3)

Table:2 IC50 Values for DPPH radical scavenging Assay by PRM and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	30.93 ± 4.178
PRM	85.01 ± 10.36

Data are given as Mean ± SD (n=3)

The trial drug was screened for DPPH radical scavenging activity and the percentage inhibition ranges from 5.912 ± 1.771 to 54.98 ± 4.249 % when compared with standard ascorbic acid with percentage inhibition ranges from 30.4 ± 4.861 to 97.38 ± 1.595 %. The IC50 value of the trial drug was found to be 85.01 ± 10.36 (µg /ml) when compared with standard ascorbic acid with (IC_{50} value 30.93 ± 4.178µg/ml)

Nitric Oxide radical scavenging assay

Table:3 Percentage inhibition of test drug PRM on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of PRM	% Inhibition of Gallic Acid
10 µg/ml	5.039 ± 2.712	27.99 ± 4.148
20 µg/ml	10.47 ± 2.572	41.57 ± 3.769

40 µg/ml	14.53 ± 3.227	53.94 ± 2.457
60 µg/ml	19.62 ± 2.519	62.21 ± 3.224
80 µg/ml	27.08 ± 2.013	83.86 ± 2.573
100 µg/ml	32.51 ± 3.616	95.36 ± 1.101

Data are given as Mean ± SD (n=3)

Table:4 IC50 Values for Nitric Oxide radical scavenging assay by PRM and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
PRM	160.1 ± 11.74
GALLIC ACID	36.64 ± 3.472

Data are given as Mean ± SD (n=3)

Nitric Oxide radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 5.039 ± 2.712 to 32.51 ± 3.616 % when compared with standard gallic acid with percentage inhibition ranging from 27.99 ± 4.148 to 95.36 ± 1.101 %. The corresponding IC50 value of the trial drug was found to be 160.1 ± 11.74 (µg /ml) when compared with standard gallic acid with (IC_{50} value 36.64 ± 3.472 µg/ml).

ABTS radical scavenging activity

Table:5 Percentage inhibition of test drug PRM on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of PRM	% Inhibition of Gallic Acid
10 µg/ml	10.88 ± 3.031	32.04 ± 3.511
20 µg/ml	22.09 ± 3.645	53.91 ± 2.763
40 µg/ml	37.52 ± 2.806	66.47 ± 1.279
60 µg/ml	49.09 ± 1.497	80.65 ± 2.092
80 µg/ml	60.71 ± 3.006	87.35 ± 0.9497
100 µg/ml	73.32 ± 1.752	98.1 ± 1.258

Data are given as Mean ± SD (n=3)

Table:6 IC50 Values for ABTS radical scavenging assay by PRM and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
PRM	63.29 ± 0.6982
GALLIC ACID	22.08 ± 3.374

Data are given as Mean ± SD (n=3)

The trial drug was screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 10.88 ± 3.031 to 73.32 ± 1.752 % when compared with standard BHA with percentage inhibition ranging from 32.04 ± 3.511 to 98.1 ± 1.258 %. The

corresponding IC50 value of the trial drug was found to be 63.29 ± 0.6982 (µg /ml) when compared with standard Gallic acid with (IC_{50} value 22.08 ± 3.374 µg/ml)

Hydrogen peroxide radical scavenging activity

Table:7 Percentage inhibition of test drug PRM on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of PRM	% Inhibition of BHA
10 µg/ml	2.967 ± 2.263	31.64 ± 3.5
20 µg/ml	7.444 ± 2.215	41.9 ± 3.279
40 µg/ml	12.09 ± 3.315	55.53 ± 2.985
60 µg/ml	17.78 ± 1.421	58.61 ± 2.919
80 µg/ml	23.91 ± 2.636	75.01 ± 2.565
100 µg/ml	27.85 ± 4.435	93.49 ± 3.937

Data are given as Mean ± SD (n=3)

Table:8 IC50 Values for Hydrogen peroxide radical scavenging assay by PRM and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
PRM	176.7 ± 14.23
BHA	36.89 ± 4.863

Data are given as Mean ± SD (n=3)

The trial drug was screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 2.967± 2.263 to 27.85± 4.435 % when compared with standard BHA with percentage inhibition ranging from 31.64 ± 3.5 to 93.49±3.937%. The corresponding IC50 value of the trial drug was found to be 176.7±14.23 (µg /ml) when compared with standard BHA with (IC_{50} value 36.89 ± 4.863 µg/ml).

CONCLUSION

Based on the results obtained from the In-vitro anti-oxidant assay for the sample PRM it was concluded that the Siddha formulation PRM has promising anti-oxidant activity in the estimated assays.

NOTE:

The study highlights the efficacy of "SIDDHA" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1) Mr.R.C.Mohan, Pulippani Vaithiyam-500, 2nd Edition, Thamarai Noolagam.
2) Thyagarajan.R. Siddha Maruthuvam Sirappu, 3rd Edition, Commissionerate of Indian Medicine and Homoeopathy, Chennai,2008.
3) Indap MM, et al; Evaluation of antioxidant and anticlastogenic properties of sponge Tetheya lynevrium [Linn]. Indian drugs No.45 aso:9 Sep:2008.
4) V.R.Patel, P.R.Patel, and S.S.Kaja, "Antioxidant activity of some selected medicinal plants in Western region of India," Advances in Biological Research, vol. 4, no. 1, pp. 23–26, 2010.
5) D.M.Nagmoti, D.K.Khatri, P.R.Juvekar, and A.R.Juvekar, "Antioxidant activity and free radical-scavenging potential of Pithecellobium dulce Benth seed extracts," Free Radical and Antioxidants, vol. 2, no. 2, pp. 37–43, 2011.
6) H. V. Bhaskar and N. Balakrishnan, "In Vitro antioxidant property of laticiferous plant species from Western Ghats Tamilnadu, India," International Journal of Health Research, vol. 2, no. 2, pp. 163–170, 2009.
7) D.K.Sahoo and A.Roy, "Compromised rat testicular antioxidant defence system by hypothyroidism before puberty," International Journal of Endocrinology, vol. 2012, Article ID 637825, 11 pages, 2012.
8) M. R. Udhayasankar, U.Danya, and K. Arumugasamy, "Phytochemistry and Free radical scavenging activity of Wattakaka volubilis (Linn. f.) Benth ex. Hook f. (Asclepiadaceae) a rare and threatened medicinal plant," International Journal of Pharm Tech Research, vol. 4, no. 3, pp. 1025–1032, 2012.
9) A. Agarwal, A. Aponte-Mellado, B.J.Premkumar, A.Shaman, and S.Gupta, "The effects of oxidative stress on female reproduction: a review," Reproductive Biology and Endocrinology, vol.10, article 49, 2012.
10) Sundarrajan, S.; Arumugam, M.A Systems Pharmacology Perspective to Decipher the Mechanism of Action of Parangichakkai Chooranam, a Siddha Formulation for the Treatment of Psoriasis. Biomed. Pharmacother. Biomede-cine Pharmacother.2017, 88,74–86.
11) Badami, Omprakash, Dongr SH,Suresh B. *In-vitro* Antioxidant property of *Solanum Pseudocapsicum* leaf extract. *Indian J Pharmacol*.2005; 37:251-252
12) B. N. Panda, A. B. Raj, N. R. Shrivastava, and A.R.Prathani, "The evaluation of nitric oxide scavenging activity of Acalypha indica Linn Root," Asian Journal Research Chemistry, vol. 2, no. 2, pp. 148–150, 2009.
13) N. Pellegrini, M. Ying, and C. Rice-Evans, "Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2′-azobis (3-ethylbenzothine-6-surfonic acid) radical cation decolorization assay," Methods in Enzymology, vol. 299, pp. 384–389, 1999.

14) Ruch R. J., Cheng S.-J., Klaunig J. E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003–1008.