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In vitro Antioxidant Activity of *Parangipattai Rasayanam* (PRM)- A Siddha Polyherbal Formulation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: The Siddha system of medicine uses a interesting combination of herbs, minerals and metals to promote good health and longevity. *Parangipattai 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.*
Aim: Evaluate the in-vitro antioxidant activity of *Parangipattai Rasayanam*.
Materials & Methods: The antioxidant activity of *Parangipattai Rasayanam* was evaluated by using various assays such as DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay, Nitric Oxide Radical Scavenging Assay, ABTS Assay, Hydrogen Peroxide Radical Scavenging Assay.
Result: *Parangipattai Rasayanam* has promising anti-oxidant activity in the estimated assays.

Keywords: Siddha medicine; polyherbal formulation; parangipattai rasayanam; kuttam; Anti-oxidant property.

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1. INTRODUCTION

Siddha system of medicine is the oldest medical systems of India that existed separately in early times. For many centuries, the system has thrived in India. Although this system has declined in later years as a result of changing lifestyles and modern medicine, it retains its influence on the masses due to its incomparable intrinsic merits. Siddha medicine can treat all types of diseases, particularly chronic diseases that confound and elude even the most sophisticated modern 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" [1]. "Kayam" which means body and "Karpam" which 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.

"During recent years, there has been a great attention to the field of free radical chemistry. Free radicals such as ROS (Reactive oxygen species) and RNS (Reactive nitrogen species) are generated by our body by different endogenous systems, exposure to different physiochemical conditions or pathological conditions. The damaging effect of free radicals causing health damages is termed oxidative stress and nitrosative stress. Excessive production of Reactive oxygen species results in oxidative stress, a harmful process that can damage cell structures, including lipids, proteins, and DNA" [3].

Balance between free radicals and antioxidants is essential for normal physiological function. If free radicals overcome the body's ability to regulate them, a condition known as oxidative stress develops. Free radicals thus adversely alter lipids, proteins, DNA and trigger many human diseases. Hence there is a need to find out a potent antioxidant drug from natural resources.

"Natural antioxidants are considered to be safe and bioactive" [4]. "Antioxidants obtained from natural sources are the only alternative to synthetic antioxidants in counteracting the free radicals connected with disease" [5]. "Plants,which are rich in phytochemical compounds, are considered as good sources of antioxidants and radical scavengers" [6]. "During recent years, many species of plants are used in the preparation of drugs and are consumed as food owing to their antioxidant activities" [7]. "So, antioxidants with free radical scavenging properties of medicinal plants may have great relevance in the preventing diseases and in therapeutic properties" [8]. "Antioxidant activity of phenolics plays a key role in the neutralization of free radicals" [9]. Antioxidant properties of phenolic compounds are mainly owing to the redox properties, which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers, in additional to their metal-chelating potential. In ancient times, this has been clearly stated in the Siddha system of medicine as Kayakarpam Therapy (Rejuvenation).

Siddha system of medicine has a holistic approach to life, balancing the mind and body with the environment and an importance on health rather than on disease [10]. *Parangipattai Rasayanam (*PRM) is a classic Siddha formulation selected 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* [11]. Phytosterols, Flavonoids, Amino acids, Terpenoids, Phenolic Compounds and Tannins, Saponins, Carbohydrates were present in *Parangipattai Rasayanam*. The main objective of the study is 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.

2. MATERIALS AND METHODS

2.1 Standard Operating Procedure of *Parangipattai Rasayanam*

2.1.1 Collection of raw drugs

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

2.2.2 Raw drugs Identification and authentication

The ingredients were identified and authenticated by Medicinal Botanist at National Institute of Siddha, Tambaram Sanatorium, Chennai.

2.1.3 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 NIS.

2.1.4 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."

2.1.5 Preparation

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

2.1.6 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 PRM was mixed with 95% methanol to prepare the stock solution in the required concentration. From the stock solution the serial dilution the concentration of 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml was made respectively" [12]. "Besides, 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" [12]. "Absorbance in the presence of test sample PRM 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" [12].

2.2 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. Moreover, 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 [13]". "Then, 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). In addition, Gallic acid was used as the positive control. The percentage inhibition of the test drug PRM and standard was calculated and recorded" [13]. 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:

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

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

2.3 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 (3ethylbenzothiazoline-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" [14]. "The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. Also, 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)" [14]. "To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of the test sample (10100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured at 734 nm. Besides, 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" [14]. The antioxidant activity of the test sample PRM was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

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

2.4 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 10100µg/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. In addition, BHA was used as the positive control" [15]. 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:

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

3. RESULTS

3.1 DPPH Radical Scavenging Activity

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).

3.2 Nitric Oxide Radical Scavenging Assay

NO radical scavenging activity of the trial drug PRM shown that the % inhibition of the test drug ranges from 5.039 ± 2.712 to 32.51 ± 3.616 % when compared with standard gallic acid with % inhibition ranging from 27.99 ± 4.148 to 95.36 ± 1.101 %. The corresponding IC50 value of the trial drug PRM 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).

3.3 ABTS Radical Scavenging Activity

The trial drug was screened for H_2O_2 radical scavenging activity and the %inhibition ranges from 10.88 ± 3.031 to 73.32 ± 1.752 % when compared to the standard BHA with % inhibition ranging from 32.04 ± 3.511 to 98.1 ± 1.258 %. The corresponding IC50 value of the trial drug PRM 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).

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)

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)

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)

3.4 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).

4. DISCUSSION

Widespread research has shown that medicinal herbs contain various active principles, which are accountable for antioxidant activity [16]. A variety of phytochemicals of antioxidant value present in medicinal herbs are responsible for this bioactivity. Qualitative phytochemical study of PRM revealed the presence of Phytosterols, Phenolic Compounds, Flavonoids, and Tannins along with other antioxidant phytochemicals. Flavonoids and Phenols, which are the most important antioxidant phytochemicals, which may have added the antioxidant potency of the trial formulation PRM.

One of the phenolic compounds found in plants are Flavonoids, and they are capable of playing the role of antioxidants that protect the cells from the damaging effects of free radicals [16,17]. The flavonoids structure, location of its hydroxyl atom, and other properties are responsible for antioxidant and reactive species neutralizing capacity [18]. These molecules reveal potent scavenging effects of destructive radicals that are connected with several disorders [19].

The antioxidant potential of phytochemicals is thought to be through the oxidative and reductive

capacity that allows absorption and neutralizing effect of free radicals [20]. Many of these secondary metabolites are endowed with significant reductive abilities that are attributed to lesser incidences of death and suffering due to oxidative stress-related disorders.

The trial formulation is used traditionally to manage many diseases, which are in association with oxidative stress. Based on the remarkable antioxidant effects demonstrated in PRM the medicinal value of this formulation could be exerted through the normalization of oxidative stress. Furthermore, this study confirms the use *of PRM* in managing oxidative stress-related diseases in Siddha system of medicine.

5. CONCLUSION

The estimated assays exhibits a promising antioxidant activity which can be confirmed from the results obtained

DISCLAIMER

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.

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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