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.

Article Information
DOI: 10.9734/JPRI/2021/v33i64B36235

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/86229

Original Research Article

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), Gunnam (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.
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 Pulippan vaithiyam-500. It is indicated for Soolai (Pain), Viranam (Various ulcers), Kiranthi (Venereal diseases), Kuttam (Skin diseases), Gunnam (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 kizhang (Curculigo orchioides) : 35grams.
Amukkara kizhang (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 (Thespisia populnea) : 35 grams.
Sengadhthari pattai (Capparis sepiaria) : 17.5grams.
Chhukku (Zingiber officinale.Roscoe.) : 17.5grams.
Thippili (Piper longum.Linn.) : 17.5 grams.
Milagu (Piper nigrum.Linn.) : 17.5 grams.
Omam (Carum coticum) : 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°C. 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 $10^{-1}$ to $100 \mu g/mL$ 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:

$\text{Radical scavenging} (\%) = \frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \times 100,$

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:

$\text{Radical scavenging} (\%) = \frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \times 100,$
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 (IC50 value 30.93 ± 4.178 μg/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 PRM was found to be 85.01 ± 10.36 (μg /ml) when compared with standard ascorbic acid with (IC50 value 30.93 ± 4.178 μg/ml).

3.3 ABTS Radical Scavenging Activity

The trial drug was screened for H₂O₂ 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 (IC50 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 (IC50 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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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/86229