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Anti oxidant activity of Siddha Herbo mineral formulation Palagarai Chunnam

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Abstract

The siddha system of medicine uses a fascinating combination of herbs, minerals and metals and to promote good health and longevity. The antioxidant activity of medicinal formulations and their roles in the prevention and treatment of various human chronic and degenerative diseases have attracted more and more attention. ***Palagarai chunnam* (PC)** is one of the Siddha formulations which are indicated as an effective drug for various diseases. The aim of the study to determine the antioxidant activity of the palagarai chunnam by using Nitric acid Radical scavenging assay, ABTS assay, DPPH assay. Based on the results obtained from the In-vitro anti-oxidant assay for the sample of PC it was concluded that the Herbo mineral formulation PC has promising anti-oxidant activity in the estimated assays.

Keywords: Palagarai chunnam, Antioxidant assay, Siddha medicine.

1. Introduction

Siddha Medical system is a special, significant and scientific system, being in practice, since time immemorial. It is one of the ancient systems of medicine contemporaneous with Grecian, Egyptian, Mesopotamian, Chinese medicines. It is a unique system which dwelt among the Tamil people of South India rendering service to humanity for more than five thousand years BC era in combating diseases and in maintaining physical, mental, social and spiritual health

Traditional Medicine has played an important role in meeting the demands of primary health care in many developing countries and its use has expanded widely in many developed countries[1]. Siddha system of medicine is one among them, which has flourished in the Southern India especially Tamilnadu[2].In Siddha system of medicine, Drugs are prepared with ingredients of herbs, minerals, metals and animal products. Siddhars use single or combinations in their medicine preparation in addition of herbs to increase its potency, efficacy, therapeutic index

because of the long shelf life. Siddhars are highly intellectual and spiritual combined with supernatural power their works involve high order of chemistry (Rasavatham). Exclusivities of Siddha system are kayakarpam, Attangayogam, Muppu, Varmam, Envagaithervu, manikkadai nool, Sarakkuvaippu, 32 types of internal medicine and 32 types of external medicine.Siddha medicine uses herbal formulations as a first line drug of choice, which emphasizes to use roots of the plants as medicine primarily and then to use leaves and other parts of the plant and finally to use herbo-mineral preparations in its oxide form, sulphide forms etc [3].Siddhas have contributed tremendous work on raw materials from herbal, herbo-mineral, metal, marine and animal origin and formulated many medicines. The unique formulations in Siddha Medicine include Parpam (mineral/metallic oxides), Chendhuram (mineral/metallic sulphides), and Chunnam (caustic or major oxides).

Palagarai chunnam is one of the traditional Siddha formulation which is indicated as a best drug for Female Infertility, Dysmennorhea, Anaemia, Dropsy in Siddha text *Siddha maruthuva nool thirattu - Anubhava Siddha Vaithiya Muraigal*. Therefore, an endeavor has been made to reveal the facts about the Herbomarine Siddha drug Palagarai chunnam, a oxide product cowry shell from the literature by scientific analysis of its purification and preparation process by evaluating the Physico chemical characters, Pharmacological actions and toxicological analysis.

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C, and vitamin E. Although certain levels of antioxidant vitamins in the diet are required for good health[4].

2. Materials and Methods

2.1 Preparation of test drug

Take the mentioned quantity of *Palagarai* **(**C*ypraea moneta*) as per text and kept immersed in juice of lemon upto 24 hours. Then wash those *Palagarai* by using water. Those purified *Palagarai* have to be kept inside the 200g of Grinded Ilaikalli (*Euphorbia nerrifolia* Linn.) Leaves and it is covered by 5 layers of mud sealed cloth and dried well. Then it will be subjected into *putam* by using 30 cow dung cakes[5]. After incineration remove the mud sealed cloth and collect the *chunnam*. Then it will be grind and have to be kept in air tight container.

Sample Solubility

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

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

The antioxidant activity of test drug sample was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Sample was mixed with 95% methanol to prepare the stock solution in required concentration (10mg/100ml or 100µg/ml). 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 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 were used as standard was prepared in same concentration as that of the sample extract by using methanol as solvent. 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 at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100 µg/ml) was noted after 15 min incubation period at 37^0C. 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 test sample 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 [6].

b) Nitric Oxide Radical Scavenging Assay

The concentrations of test sample 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% naphthylethylenediaminedihydrochloride 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 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 and standard was calculated and recorded [7]. The percentage nitrite radical scavenging activity of the test drug 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.

c) ABTS Assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug 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 test sample (10-100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug was measured following the same procedures described above and was used as positive controls[8]. The antioxidant activity of the test sample was calculated using the following equation:

The ABTS scavenging effect was measured using the following formula:

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

d) Hydrogen peroxide radical scavenging activity

The percentage of hydrogen peroxide scavenging of Test drug PC and standard compounds were calculated: Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4) and compared with Butylated hydroxyanisole (BHA) standard.

3. Results

1. Percentage inhibition of test drug PC on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of PC	% Inhibition of Ascorbic Acid
10 µg/ml	4.141 ± 0.75	37.95 ± 2.23
20 µg/ml	9.639 ± 0.57	49.88 ± 3.92
40 µg/ml	15.25 ± 0.41	58.45 ± 7.44
60 µg/ml	18.58 ± 0.73	66.66 ± 5.74
80 µg/ml	23.85 ± 3.86	73.74 ± 2.23
100 µg/ml	29.34 ± 3.27	88.99 ± 0.23

Data are given as Mean ± SD (n=3)

IC50 Values for DPPH radical scavenging Assay by PC and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	22.44 ± 2.32
PC	181.4 ± 28.06

Data are given as Mean ± SD (n=3)

2. Percentage inhibition of test drug PC on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of PC	% Inhibition of Gallic Acid
10 µg/ml	7.963 ± 2.25	17.13 ± 1.84
20 µg/ml	13.75 ± 3.48	36.88 ± 12.18
40 µg/ml	19.53 ± 1.87	52.19 ± 5.46
60 µg/ml	27.49 ± 1.65	63.51 ± 5.63
80 µg/ml	32.19 ± 1.67	74.85 ± 5.37
100 µg/ml	41.59 ± 4.89	85.44 ± 4.44

Data are given as Mean ± SD (n=3)

IC50 Values for Nitric Oxide radical scavenging assay by PC and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
PC	127.7 ± 15.69
GALLIC ACID	44.4 ± 7.729

Data are given as Mean ± SD (n=3)

3. Percentage inhibition of test drug PC on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of PC	% Inhibition of Gallic Acid
10 µg/ml	5.58 ± 1.17	40.54 ± 3.68
20 µg/ml	12.41 ± 0.56	57.92 ± 3.67
40 µg/ml	20.73 ± 1.21	67.13 ± 7.37
60 µg/ml	28.33 ± 0.50	73.94 ± 5.81
80 µg/ml	33.01 ± 0.46	84.17 ± 3.28
100 µg/ml	43.59 ± 1.20	97.52 ± 0.21

Data are given as Mean ± SD (n=3)

IC50 Values for ABTS radical scavenging assay byPC and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
PC	117.4 ± 1.527
GALLIC ACID	15.22 ± 9.046

Data are given as Mean ± SD (n=3)

4.Percentage inhibition of test drug PC on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of PC	% Inhibition of BHA
10 µg/ml	1.473 ± 0.76	37.65 ± 2.88
20 µg/ml	8.445 ± 0.55	50.47 ± 2.00
40 µg/ml	12.79 ± 1.53	61.69 ± 1.92
60 µg/ml	16.63 ± 0.88	72.27 ± 2.54
80 µg/ml	22.62 ± 1.90	80.28 ± 2.42
100 µg/ml	25.17 ± 1.15	87.65 ± 1.92

Data are given as Mean ± SD (n=3)

IC50 Values for Hydrogen peroxide radical scavenging assay by PC and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
PC	196 ± 13.91
BHA	23.21 ± 3.25

Data are given as Mean ± SD (n=3)

3.1 Observation

a) DPPH radical scavenging activity

Trial drug were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 4.14 to 29.34 % when compared with standard ascorbic acid with percentage inhibition ranges from 37.95to 88.99 %. The IC50 value of the trial drug was found to be 181.4 (µg /ml) when compared with standard ascorbic acid with (IC_{50}value 22.44µg/ml)

b) NO radical scavenging activity

NO radical scavenging activity of the trial drug revealed that the percentage inhibition of the test drug ranges from 7.96 to 41.59 % when compared with standard gallic acid with percentage inhibition ranges from 17.13 to 85.44 % .The corresponding IC50 value of the trial drug was found to be 127.7(µg /ml) when compared with standard gallic acid with (IC_{50}value 44.4µg/ml)

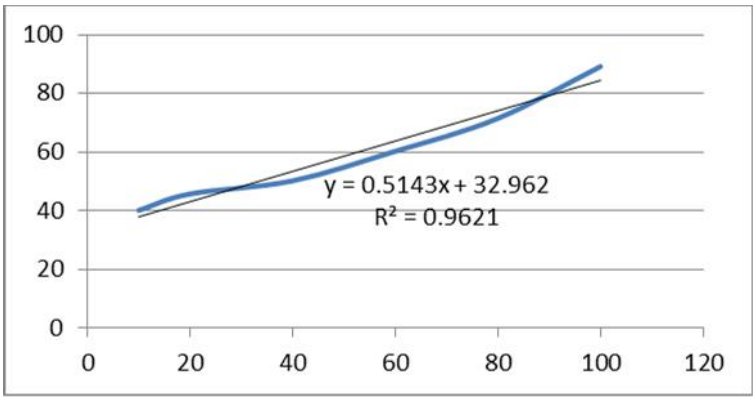
c) ABTS radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 5.58 to 43.59 % when compared with standard gallic acid with percentage inhibition ranges from 40.54 to 97.52 % .The corresponding IC50 value of the trial drug was found to be 117.4 (µg /ml) when compared with standard gallic acid with (IC_{50}value 15.22µg/ml)

d) Hydrogen peroxide radical scavenging activity

Trial drug were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 1.47 to 25.17 % when compared with standard BHA with percentage inhibition ranges from 37.65 to 87.65 % .The corresponding IC50 value of the trial drug was found to be 196 (µg /ml) when compared with standard BHA with (IC_{50}value 23.21µg/ml).

Percentage inhibition of STD on DPPH radical scavenging assay
Triplicate 1



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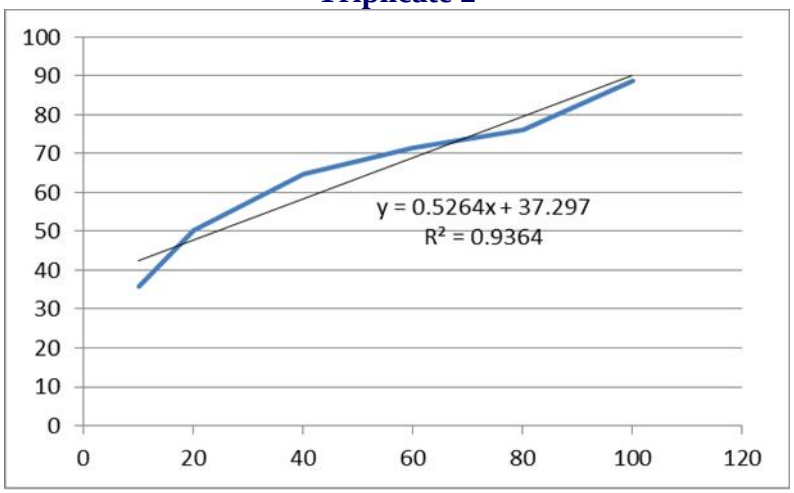

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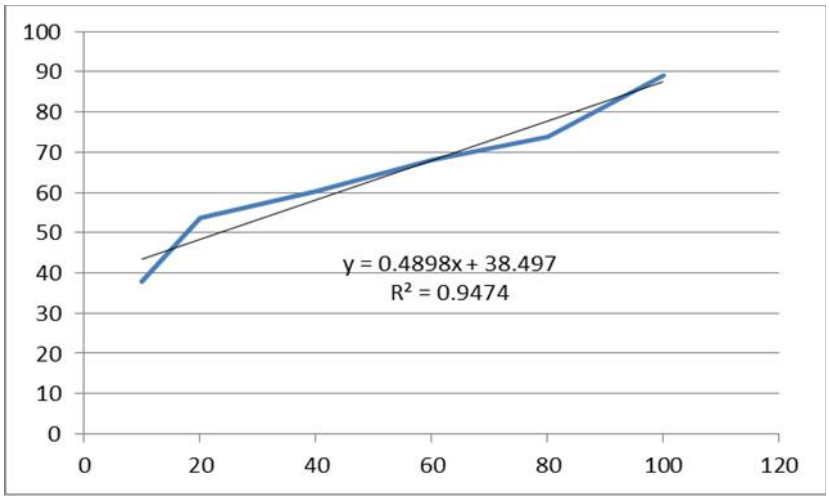

Percentage inhibition of PC on DPPH radical scavenging assay

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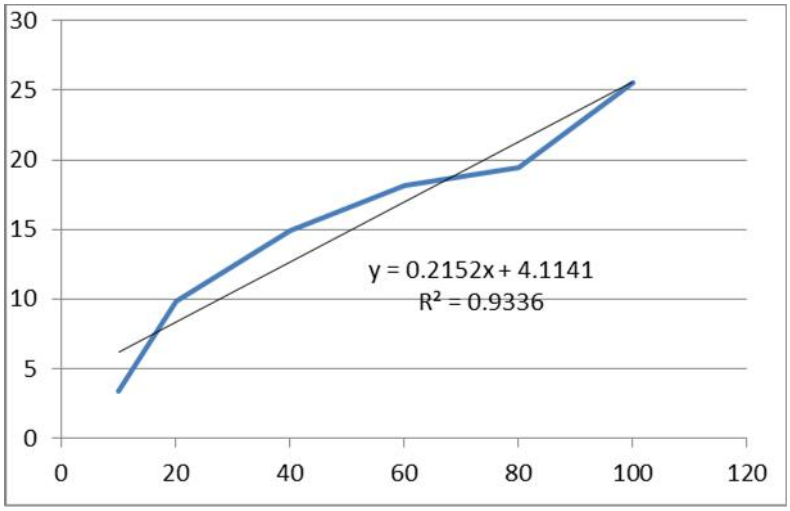

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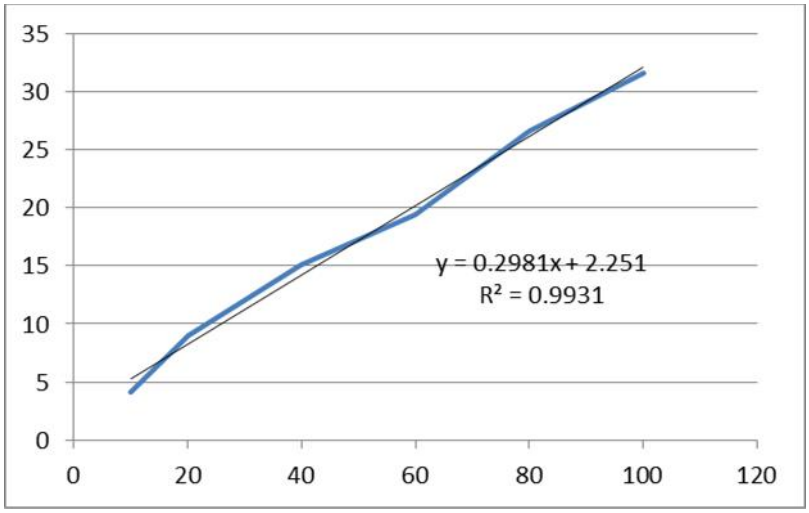

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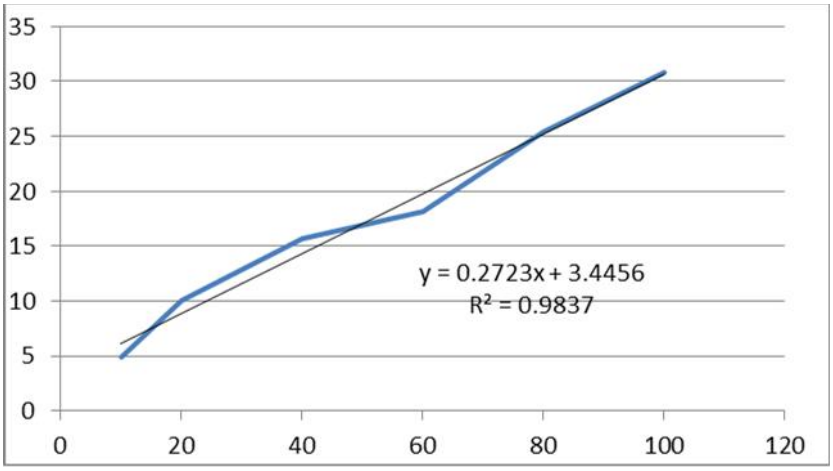

Percentage inhibition of STD on Hydrogen Peroxide radical scavenging assay

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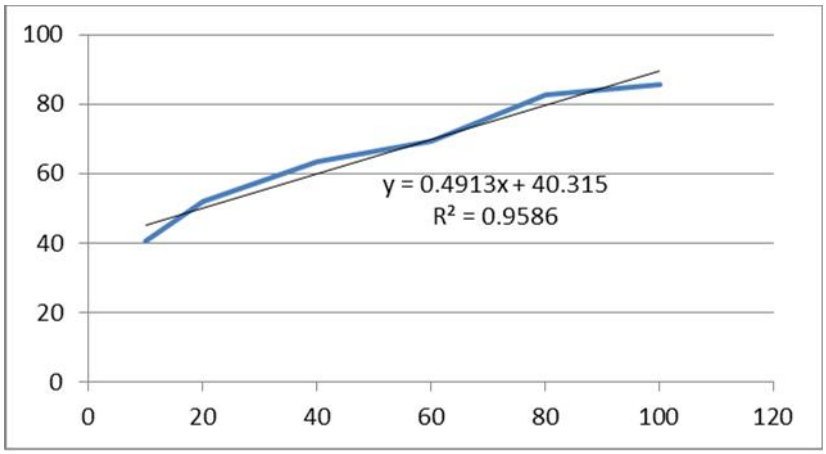

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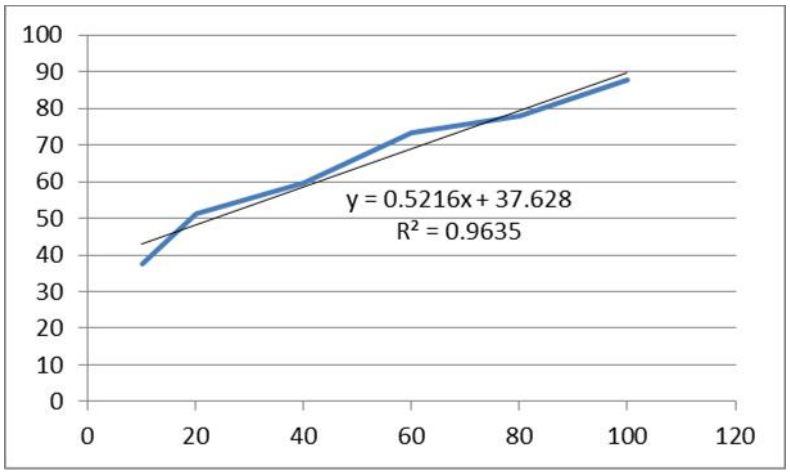

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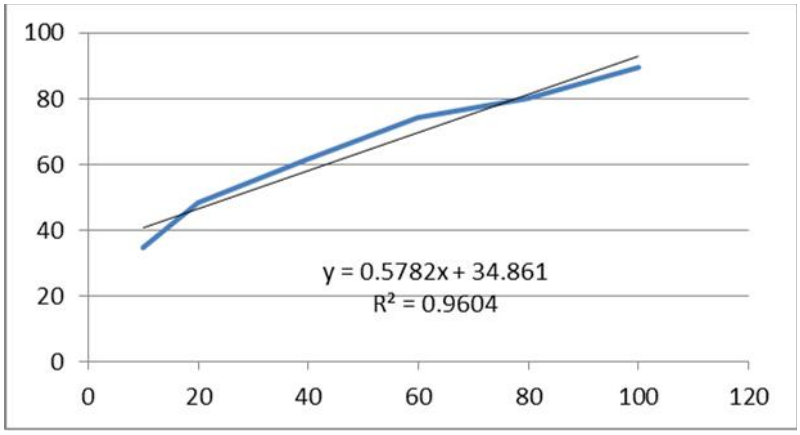

Percentage inhibition of PC on Hydrogen Peroxide radical scavenging assay

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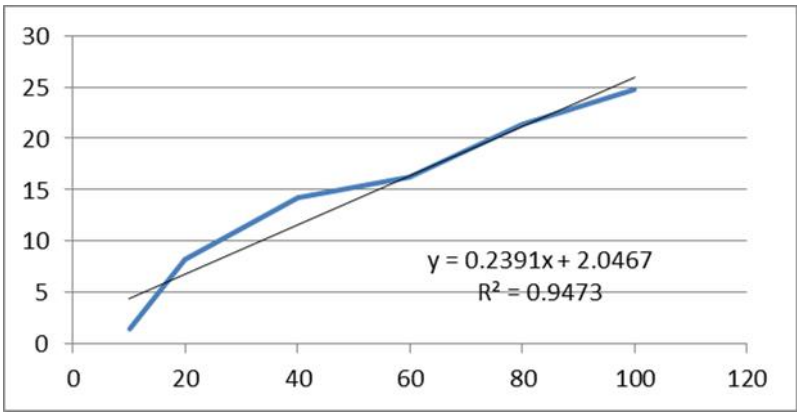

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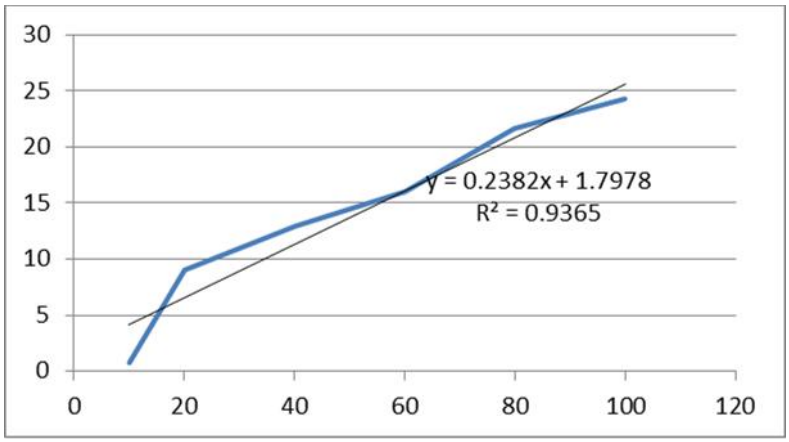

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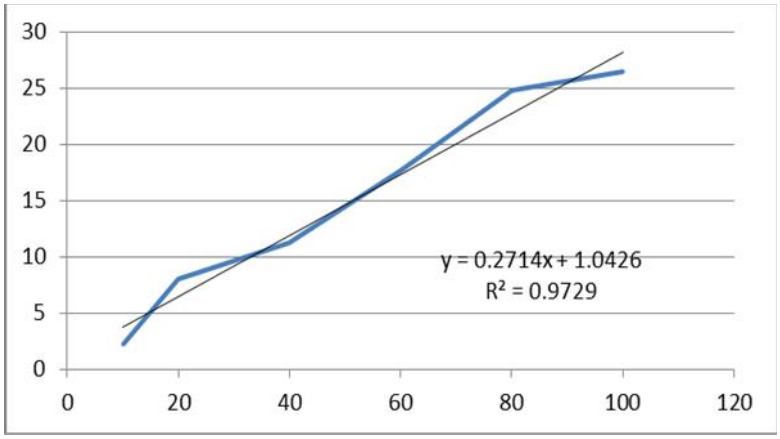

Percentage inhibition of STD on Nitric Oxide radical scavenging assay

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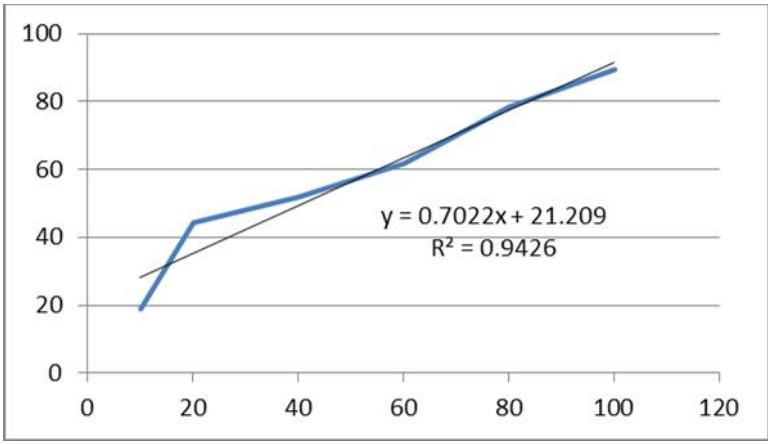

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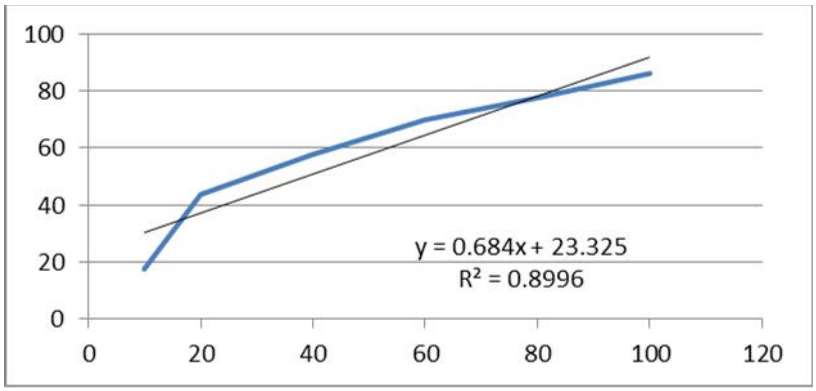

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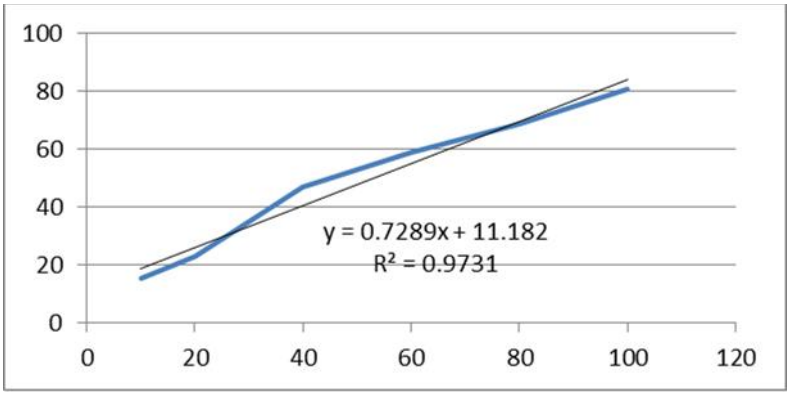

Percentage inhibition of PC on Nitric Oxide radical scavenging assay

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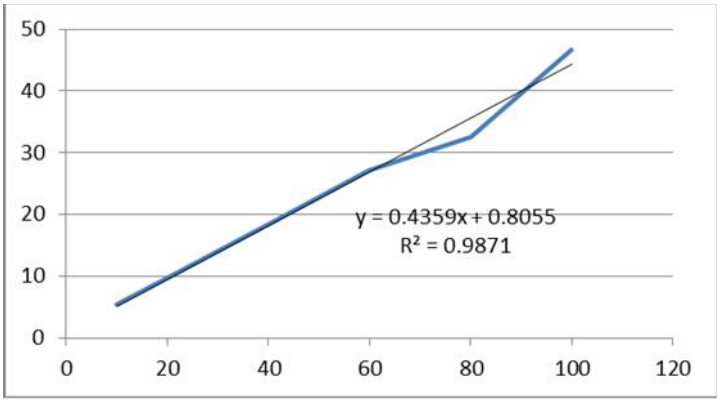

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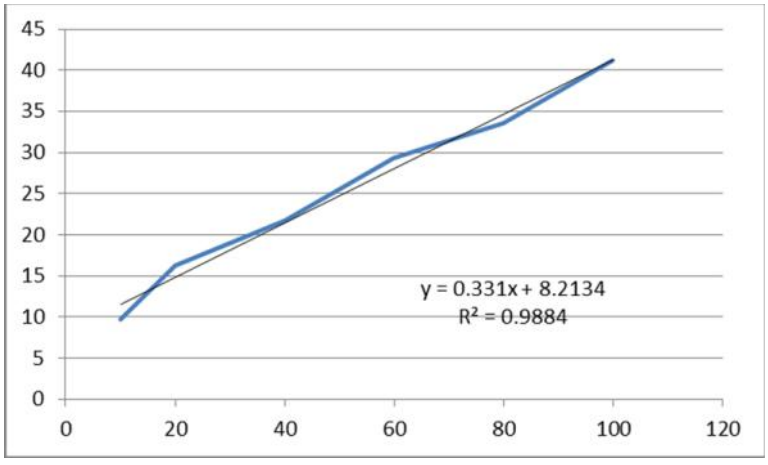

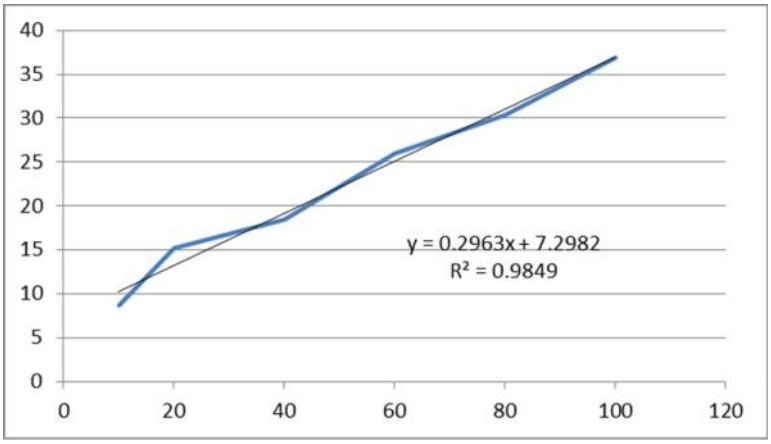

Percentage inhibition of STD on ABTS radical scavenging assay

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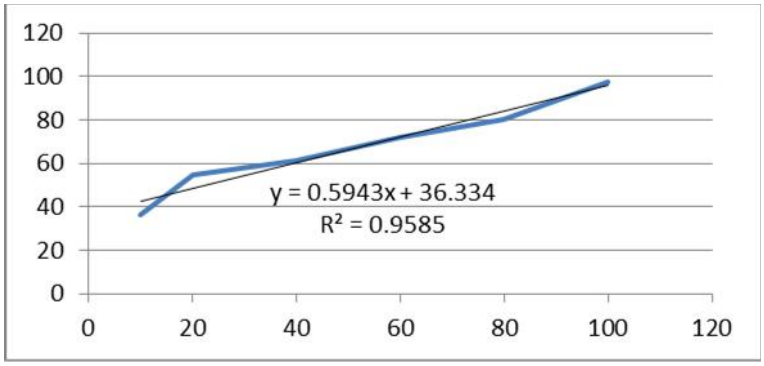

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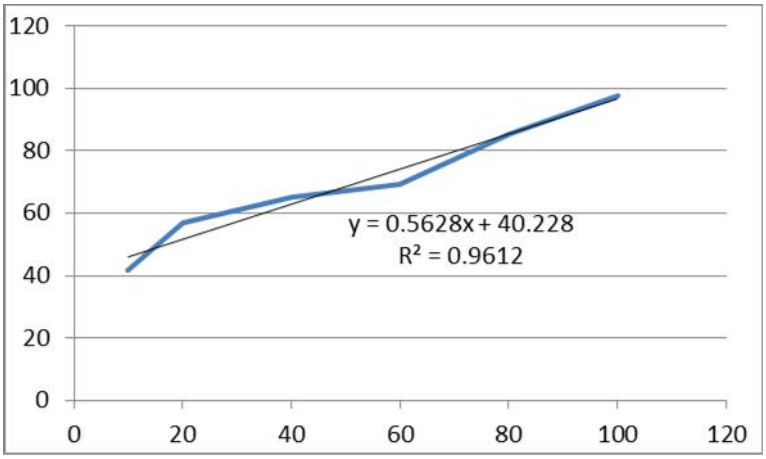

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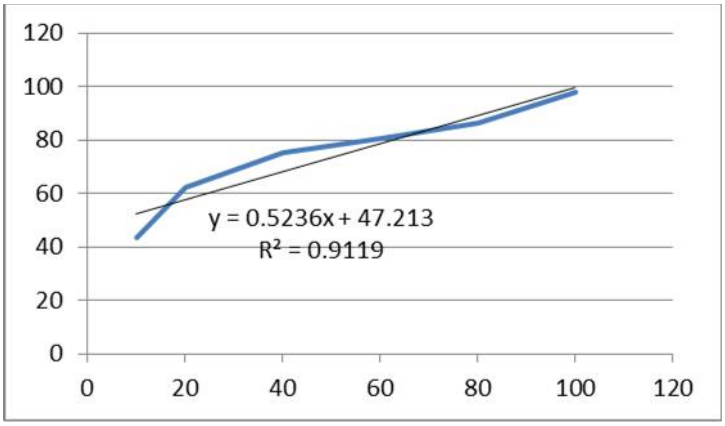

Percentage inhibition of PC on ABTS radical scavenging assay

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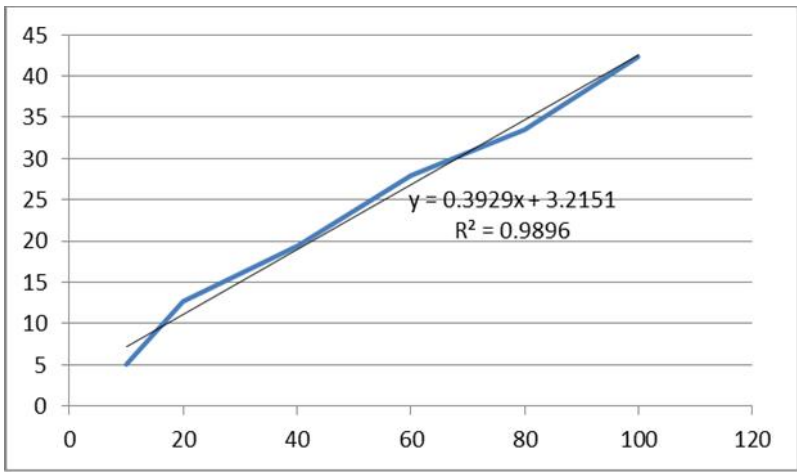

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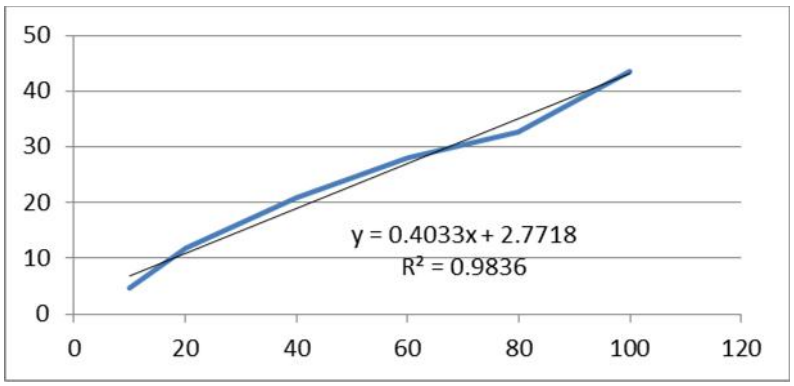

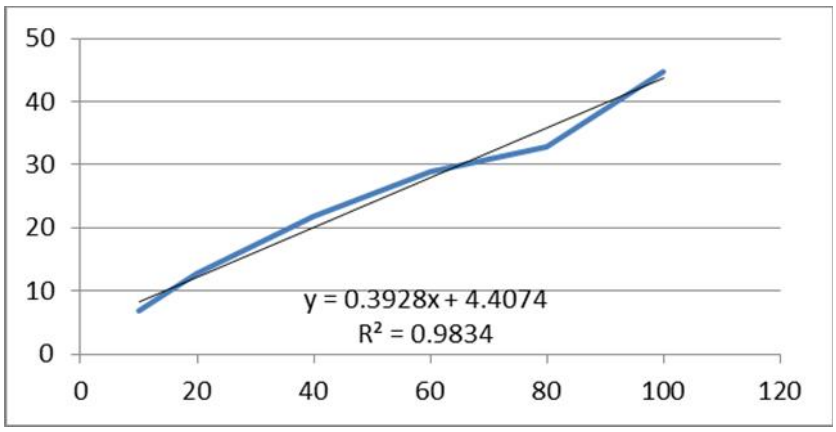

4. Discussion

These antioxidant compounds have a promising pharmacological agent for preventing cancer, cardiovascular diseases, inflammatory disorders, neurological degeneration, wound healing, infectious diseases and aging as well as it can be used as food preservatives. Potential source of natural antioxidant that could have great importance as therapeutic agents.

5. Conclusion

Based on the results obtained from the In-vitro anti-oxidant assay for the sample PC it was concluded that the siddha formulation Palagarai chunnam has promising anti-oxidant activity in the estimated assays. It is clear that the antioxidant properties of Palagarai chunnam due to the presence of some antioxidant compounds such as Vitamin C, monophenolics, flavonoids, and polyphenolics.

6. Acknowledgements

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