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ANTIOXIDANT ACTIVITY OF SIDDHA HERBO MINERAL FORMULATION OF RATHI NAGARA RASA MEZHUGU

Dr. Veerasivaraman M.[1*], Dr. Brunda S.[2], Dr. Anbarasan B.[3] and Dr. Rasakumar R.[4]

[1]PG. Scholar, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai.

[2]PG. Scholar, PG Dept. of Maruthuvam, Govt. Siddha Medical College, Chennai.

[3]Siddha Consultant, Dr Jay's Ayush Hospital, Chennai.

[4]PG. Scholar, PG Dept. of Sirappu Maruthuvam, Govt. Siddha Medical College, Chennai.

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***Corresponding Author**
Dr. Veerasivaraman M.
PG. Scholar, Dept. of Nanju Maruthuvam, National Institute of Siddha, Chennai.

ABSTRACT

Siddha system of medicine is a renowned holistic system of traditional medicine emphasizing curative and preventative measures. The medicine used in Siddha are of plant origin, metals, minerals, and animal products. Most of the Siddha medicine has improvise the longevity of life through their anti–oxidant properties. 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. The drug "*Rathi nagara rasa mezhugu*" (RNM) is a herbo mineral formulation mentioned in the Siddha literature "Anuboga vaithya navaneetham part V" indicated for Penile cancer, Cervix cancer, Cervical adenitis, Leprosy, Syphilis, Rheumatoid Arthritis. 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 RNM based on various in vitro assays. The study results confirmed that the drug RNM has promising therapeutic antioxidant activity when compared with the standard drug. This research work can help for medical practitioners to use this herbo-mineral compound for the treatment of cancer.

KEYWORDS: Rathi nagara rasa mezhugu, Anti-oxidant assay, DPPH, Siddha.

INTRODUCTION

Oxidative stress has been identified as the root cause of the development and progression of several diseases. Supplementation of exogenous antioxidants or boosting endogenous antioxidant defenses of the body is a promising way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage. Herbs have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage. Several in vitro methods have been used to screen plants for their antioxidant potential, and in most of these assays they revealed potent antioxidant activity. Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate.[1] 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.[2] So antioxidant present in the siddha medicine can protect against oxidative damage by decreasing the number of free radicals which cause chronic diseases and aging process and many other diseases. Siddhars have formulated different types of drug with antioxidant property one among such herbo mineral formulation drug "*Rathi nagara rasa mezhugu*" is mentioned in the Siddha literature "Anuboga vaithya navaneetham part V" indicated for Penile cancer, Cervix cancer, Cervical adenitis, Leprosy, Syphilis, Rheumatoid Arthritis.[3]

2. MATERIALS AND METHODS

2.1 Preparation of drug

Ingredients of Rathi nagara rasa mezhugu

- Sitramanakkennai (Castor oil) - ¼ palam (8.75 gm)
- Gandhakam (Sulphur) - 1 palam (35 gm)
- Serankottai (*Semecarpus Anacardium*) - 30 Nos
- Vaalai rasam (Mercury) - 1 palam (35 gm)
- Pasu nei (Ghee) - 1 palam (35 gm)
- Panai vellam (Palm jaggery) - 2 palam (35 gm)

Collection and identification of drug

Ingredients of the drug are bought in Ramasamy chetty shop, country traditional raw herbal drugs shop, Parrys, Chennai and are authenticated at National Institute of Siddha, Tambaram sanatorium, Chennai.

PREPARATION

Step 1

First castor oil is taken in a vessel and heated. Then Purified sulphur is powdered and mixed with the heating castor oil. When the sulphur melts, Semecarpus seeds are cut into two pieces and put in the oil. Then thailam is taken when semecarpus seeds turns red and floats. The thailam is called ***Rathi nagara thailam***.

Step 2

Mercury and Rathinagara thailam is mixed and grinded. When mercury is grinded well, ghee and palm jaggery is added and grinded to get *Rathi nagara rasa mezhugu.*

Sample Solubility

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

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

The antioxidant activity of test drug Rathi nagara rasa Mezhugu (RNM) was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. RNM 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 was 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.[4]

% 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.

b) Nitric Oxide Radical Scavenging Assay

The concentrations of test drug Rathi nagara rasa Mezhugu (RNM) 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% naphthylethylenediamine 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 RNM (10−100 µg/mL) and incubated at 25°C for 180 mins. The test drug RNM 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 RNM and standard was calculated and recorded.[5] The percentage nitrite radical scavenging activity of the test drug and gallic acid were calculated using the following formula.

percentage nitrite radical scavenging activity:

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

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

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 RNM (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.[6] 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 RNM 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 (100µg/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.[7]

3. RESULTS

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

Concentration (µg/ml)	% Inhibition of RNM	% Inhibition of Ascorbic Acid
10 µg/ml	19.07 ± 0.90	28.23 ± 4.11
20 µg/ml	32.43 ± 1.41	53.23 ± 5.98
40 µg/ml	43.8 ± 0.700	59.39 ± 6.78
60 µg/ml	52.18 ± 0.72	66.64 ± 8.23
80 µg/ml	67.44 ± 0.55	73.88 ± 9.74
100 µg/ml	71.28 ± 0.87	91.71 ± 0.22

Data are given as Mean ± SD (n=3)

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

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	30.72 ± 8.08
RNM	55.71 ± 0.46

Data are given as Mean ± SD (n=3)

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

Concentration (µg/ml)	% Inhibition of RNM	% Inhibition of Gallic Acid
10 µg/ml	4.639 ± 2.38	18.9 ± 8.50
20 µg/ml	10.74 ± 6.96	38.58 ± 6.68
40 µg/ml	16.84 ± 6.70	52.31 ± 6.13
60 µg/ml	22.95 ± 5.40	60.1 ± 9.00
80 µg/ml	27.52 ± 5.15	70.87 ± 7.79
100 µg/ml	34.39 ± 4.62	83.01 ± 8.71

Data are given as Mean ± SD (n=3)

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

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
RNM	149.7 ± 19.77
GALLIC ACID	28.92 ± 2.07

Data are given as Mean ± SD (n=3)

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

Concentration (µg/ml)	% Inhibition of RNM	% Inhibition of Gallic Acid
10 µg/ml	27.78 ± 1.12	36.68 ± 5.70
20 µg/ml	32.66 ± 0.67	47.06 ± 3.06
40 µg/ml	43.49 ± 0.88	55.76 ± 2.52
60 µg/ml	57.12 ± 0.70	64.46 ± 4.60
80 µg/ml	68.4 ± 4.08	73.16 ± 5.15
100 µg/ml	76.19 ± 0.60	94.14 ± 5.02

Data are given as Mean ± SD (n=3)

IC50 Values for ABTS radical scavenging assay By RNM and standard.

Test Drug / Standard	IC50 Value ABTS Assay ± SD (µg /ml)
RNM	50 ± 1.03
GALLIC ACID	30.56 ± 5.49

Data are given as Mean ± SD (n=3)

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

Concentration (µg/ml)	% Inhibition of RNM	% Inhibition of BHA
10 µg/ml	30.01 ± 1.39	33.41 ± 5.78
20 µg/ml	40.94 ± 3.35	54.57 ± 4.14
40 µg/ml	48.29 ± 3.25	66.6 ± 2.27
60 µg/ml	53.54 ± 3.20	73.89 ± 5.78
80 µg/ml	58.78 ± 2.45	81.04 ± 7.45
100 µg/ml	64.36 ± 5.10	89.58 ± 1.59

Data are given as Mean ± SD (n=3)

IC50 Values for Hydrogen peroxide radical scavenging assay By RNM and standard.

Test Drug / Standard	IC50 Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
RNM	54.31 ± 8.23
BHA	21.44 ± 2.78

Data are given as Mean ± SD (n=3)

Percentage inhibition of STD on DPPH radical scavenging assay

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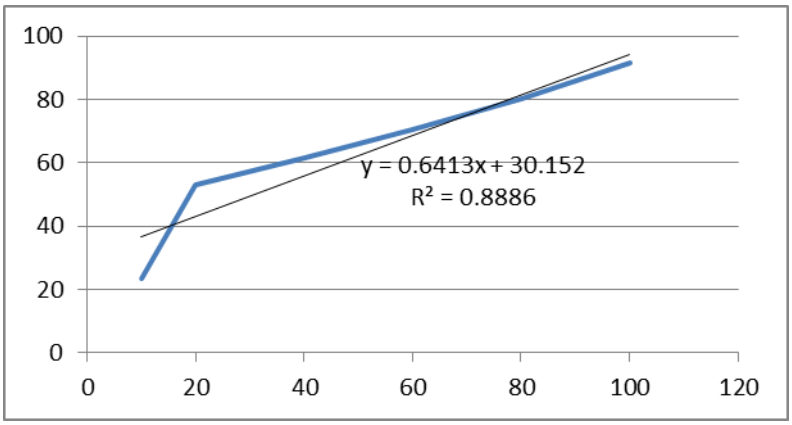

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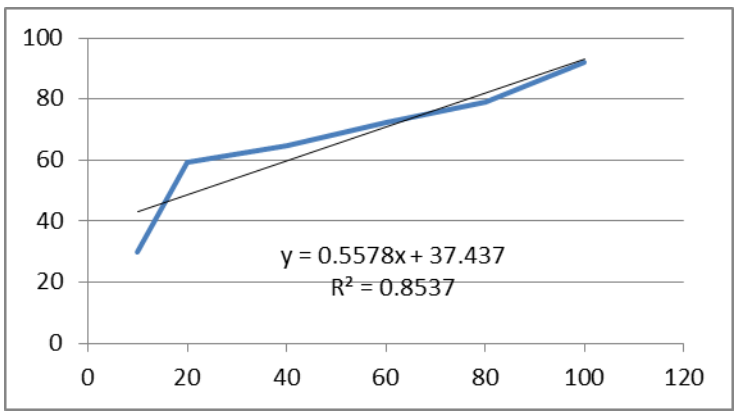

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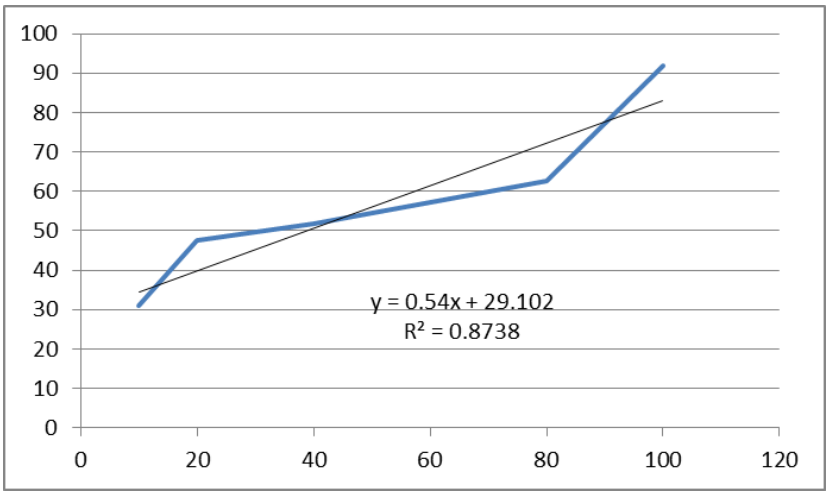

Percentage inhibition of RNM on DPPH radical scavenging assay

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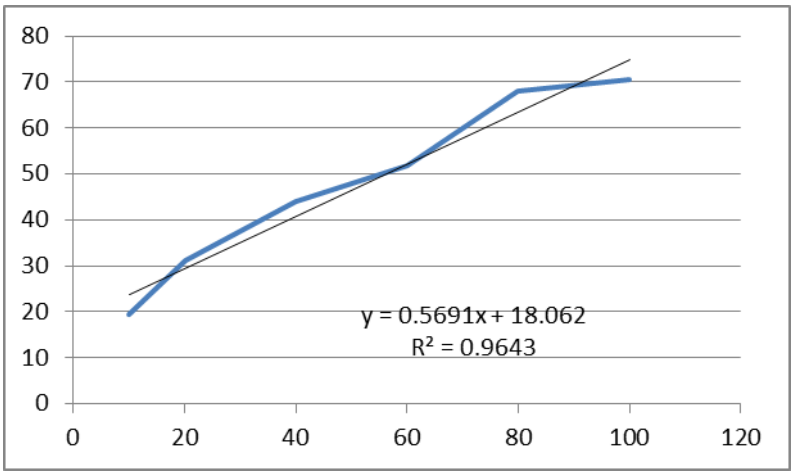

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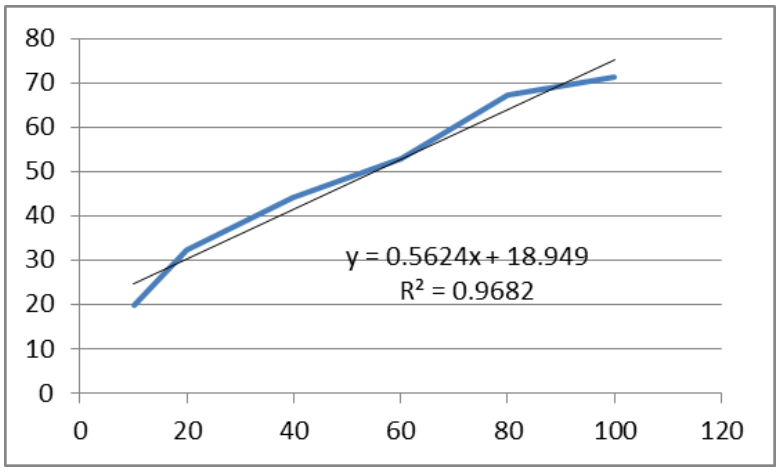

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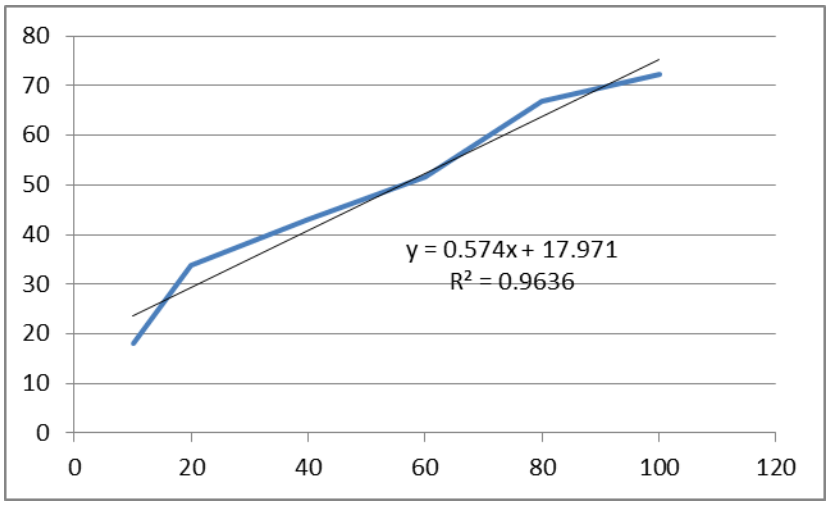

Percentage inhibition of STD on Hydrogen Peroxide radical scavenging assay

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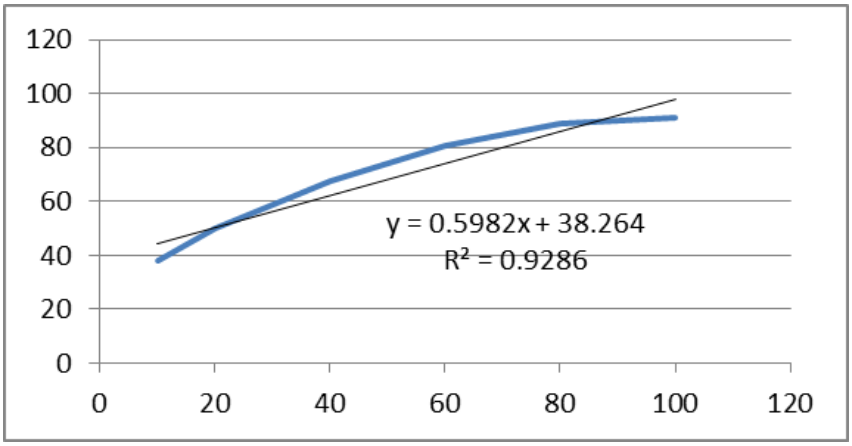

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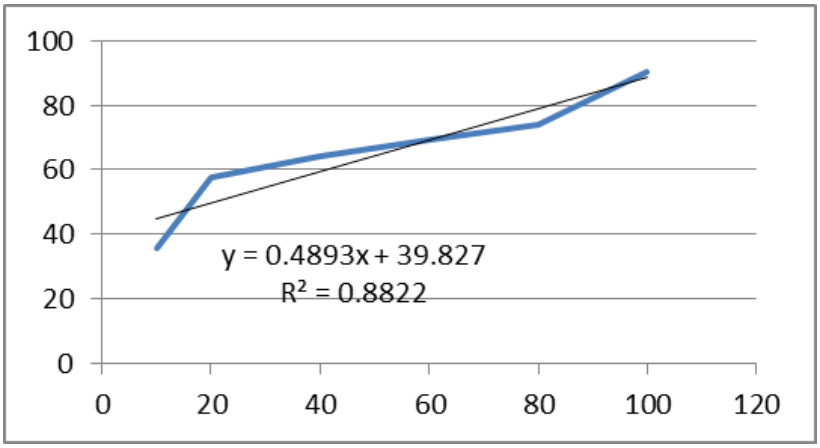

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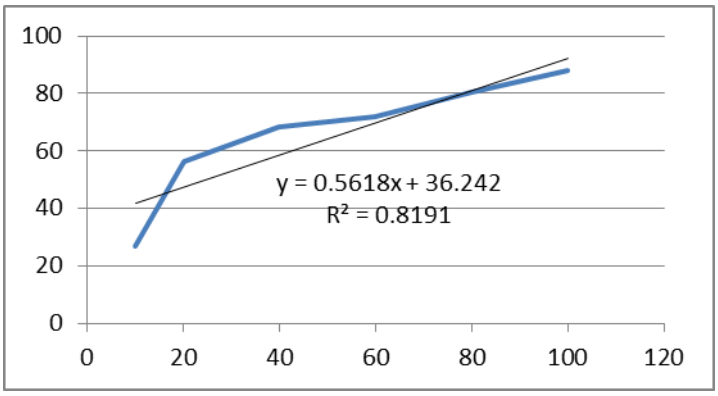

Percentage inhibition of RNM on Hydrogen Peroxide radical scavenging assay

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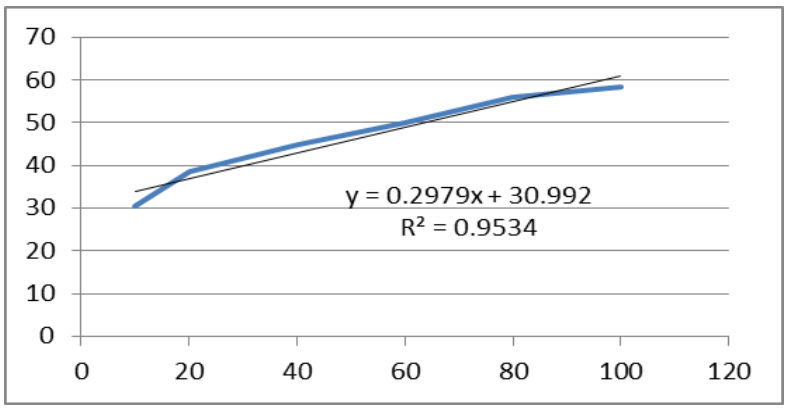

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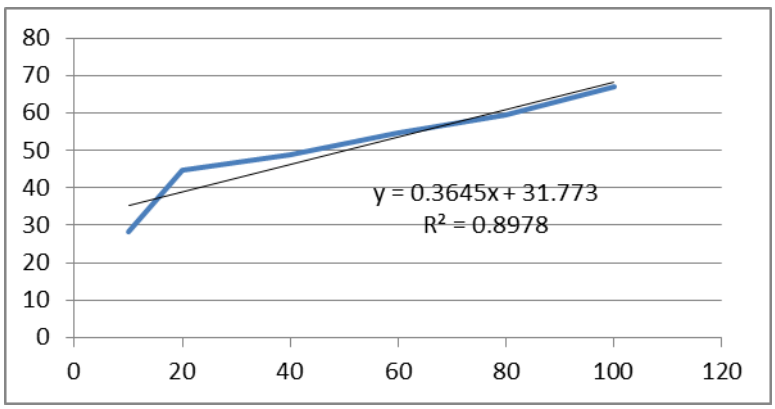

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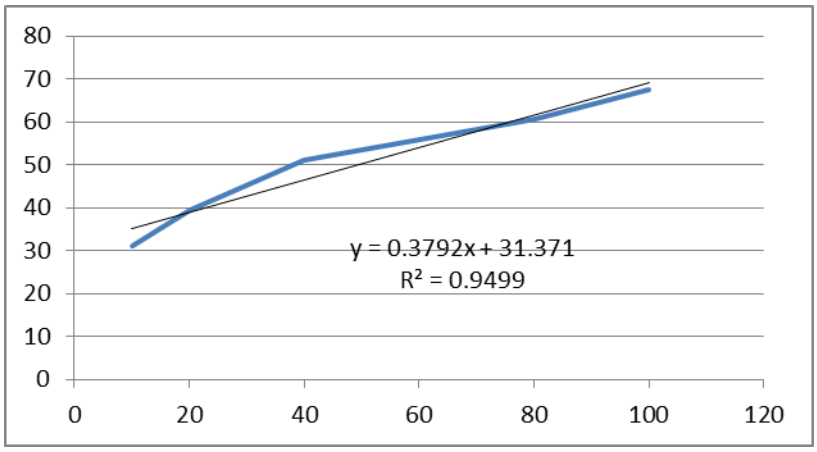

Percentage inhibition of STD on Nitric Oxide radical scavenging assay

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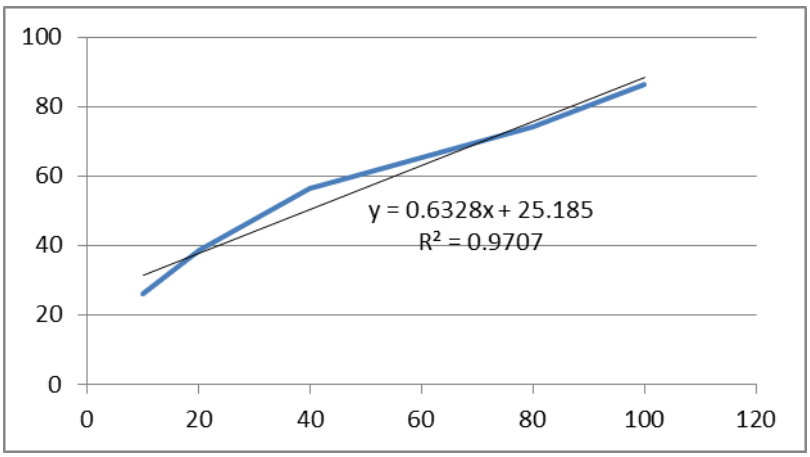

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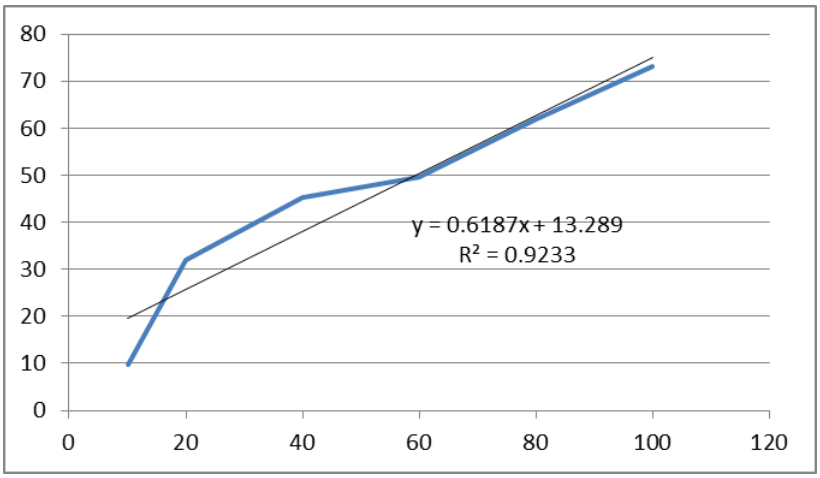

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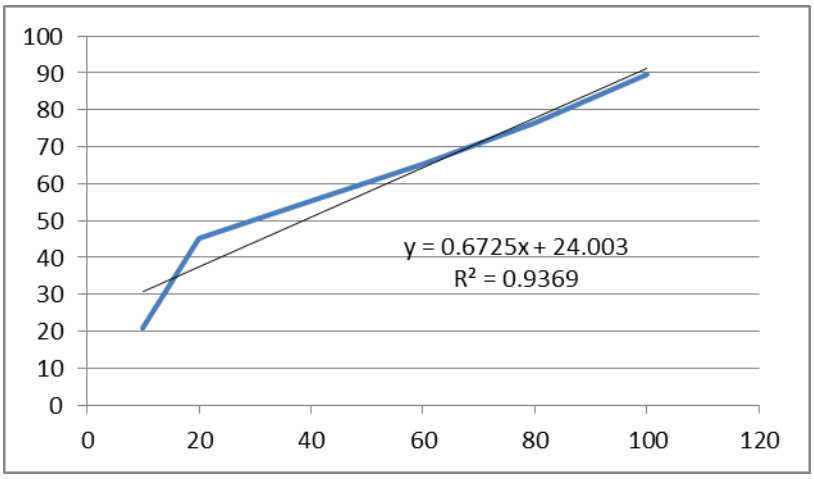

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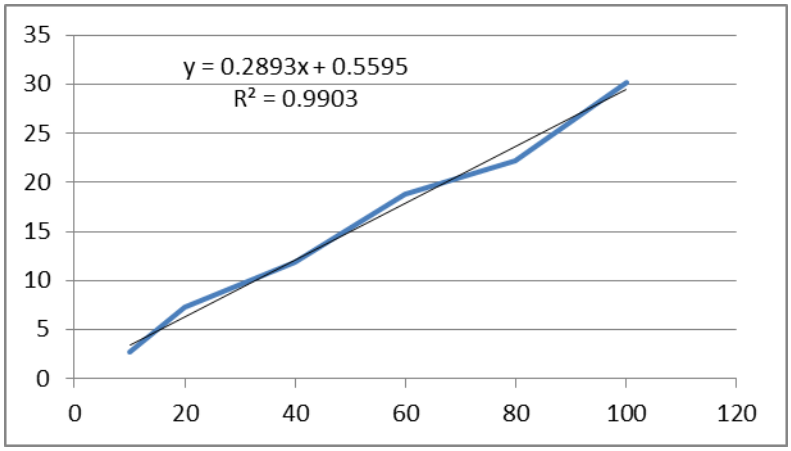

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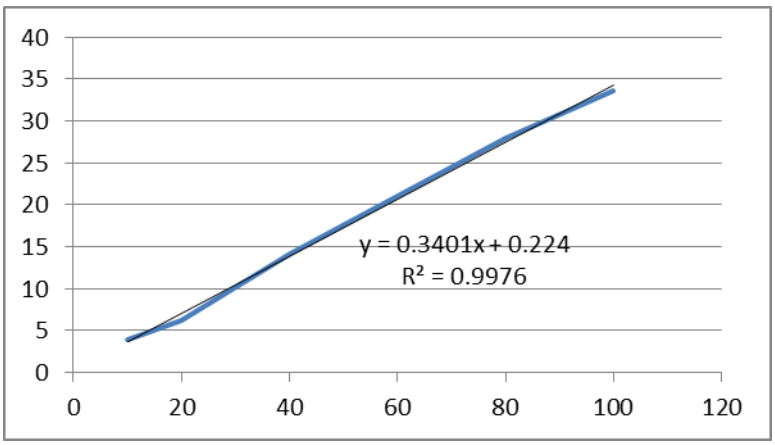

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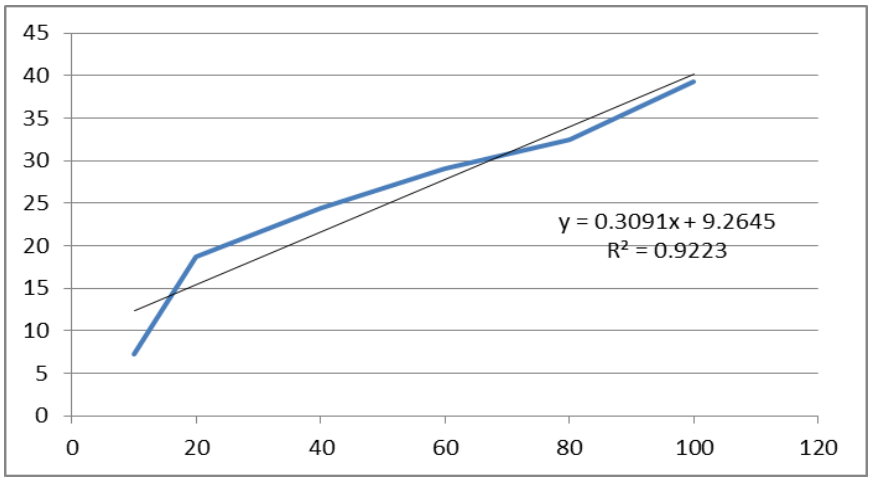

Percentage inhibition of STD on ABTS radical scavenging assay

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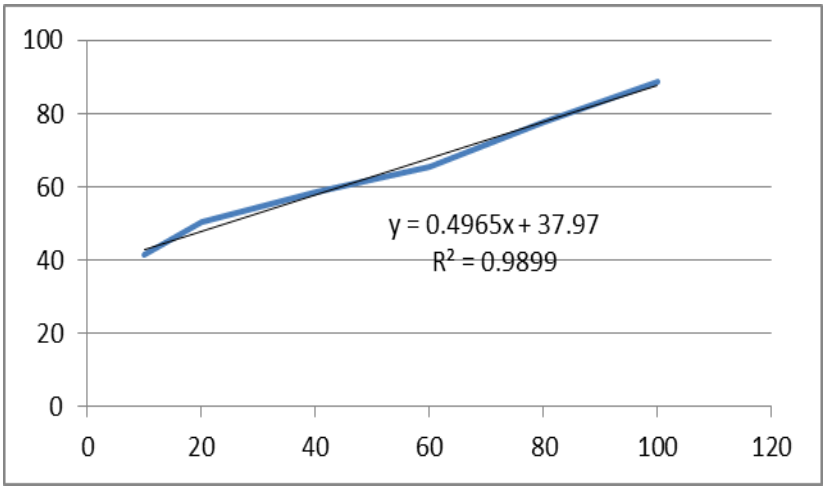

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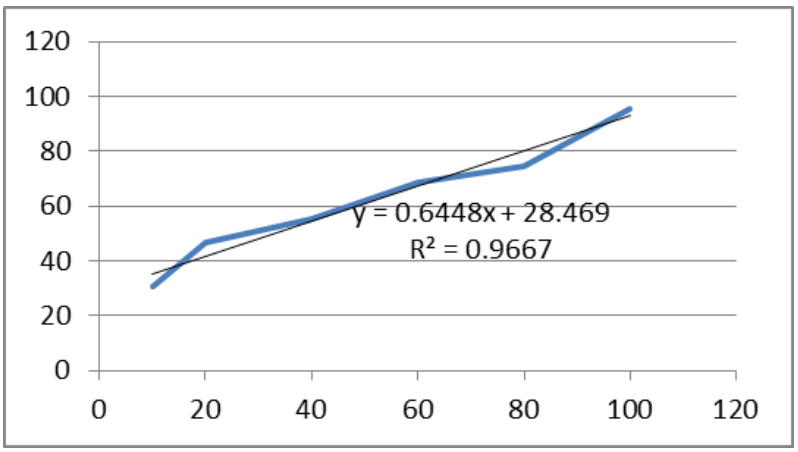

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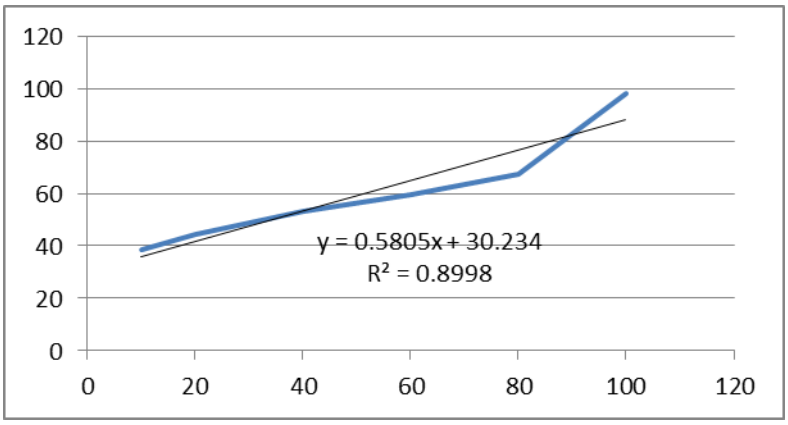

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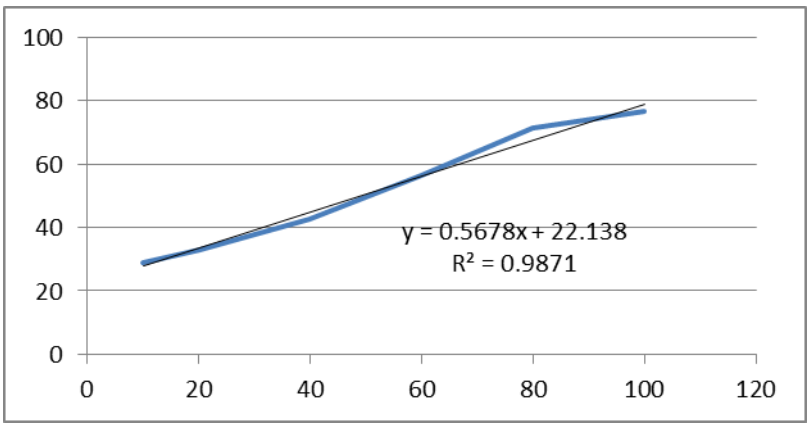

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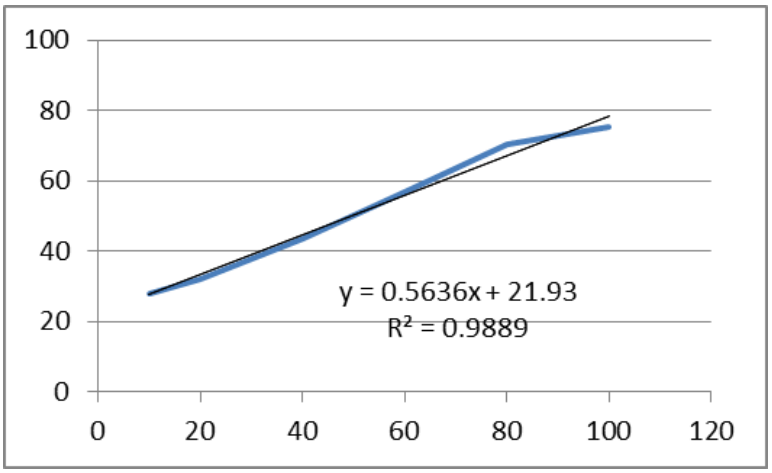

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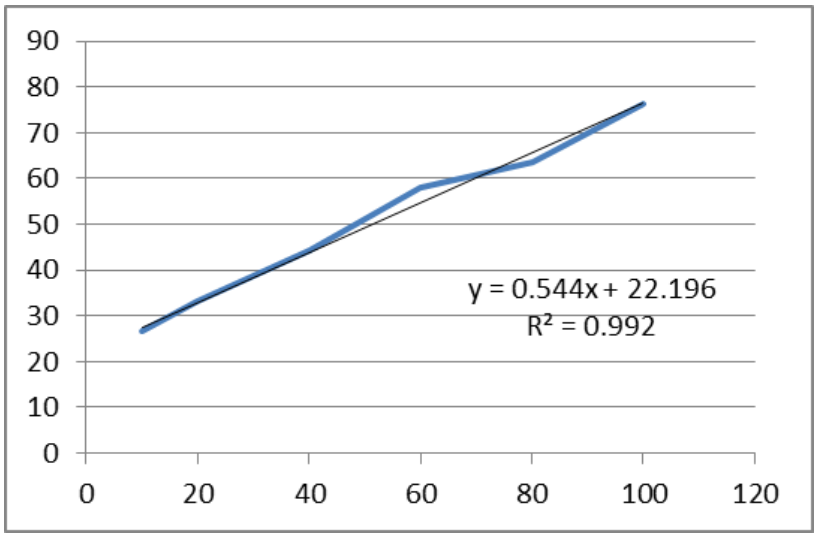

DISCUSSION

Rathi nagara rasa mezhugu (RNM) were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 19.07 to 71.28% when compared with standard ascorbic acid with percentage inhibition ranges from 28.23to 91.71%. The IC50 value of the trial drug was found to be 55.71 (µg /ml) when compared with standard ascorbic acid with (IC_{50}value 30.72µg/ml). NO radical scavenging activity of the Rathi nagara rasa mezhugu (RNM) revealed that the percentage inhibition of the test drug ranges from 4.63 to 34.39% when compared with standard gallic acid with percentage inhibition ranges from 18.9 to 83.01%. The corresponding IC50 value of the trial drug was found to be 149.7(µg /ml) when compared with standard gallic acid with (IC_{50}value 28.92µg/ml). Rathi nagara rasa mezhugu (RNM) were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 27.78 to 76.19% when compared with standard gallic acid with percentage inhibition ranges from 36.68 to 94.14%. The corresponding IC50 value of the trial drug was found to be 50 (µg /ml) when compared with standard gallic acid with (IC_{50}value 30.56 µg/ml). Rathi nagara rasa mezhugu (RNM) were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 30.1 to 64.36% when compared with standard BHA with percentage inhibition ranges from 33.41 to 89.58%. The corresponding IC50 value of the trial drug was found to be 54.31 (µg /ml) when compared with standard BHA with (IC_{50}value 21.44µg/ml).

CONCLUSION

Based on the results obtained from the In-vitro antioxidant assay of sample RNM it is evident that the siddha formulation '*Rathi nagara rasa mezhugu'* has significant antioxidant activity in the estimated assays. Besides, the antioxidant property of *Rathi nagara rasa mezhugu* is due to the presence of some antioxidant compounds such as Vitamin C, monophenolics, flavonoids, and polyphenolics.

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