Research Article

The *in vitro* Antioxidant Studies of One Ayurvedic Medicine "Sarawatharihtam."

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ABSTRACT

Sarawatharshtam is a standard Ayurvedic tonic for treating mental disorders in general. It contains plants such as Aswagandha, Bramhi and Satavari, which are classified as Medhya rasayanas i.e. medicines pertaining to mental health. The present study deals with the in vitro antioxidant assays, namely, DPPH method, Hydrogen-peroxide scavenging activity, Ferric Thyocynate method, Hydroxyl radical scavenging activity and ABTS Assay methods of Saraswatharishtam. It was observed that all the assays indicated fair levels of antioxidant activities when compared to the standards.

Keywords: Saraswatharishtam, Ayurvedic, Aswagandha, Bramhi, Satavari, DPPH, ABTS.

INTRODUCTION

Ayurvedic and Sidhha medicines have come of age as methods of treatment in India. But due to the lack of scientific and pharmacological studies these systems of medicine are not getting the due recognition. It is highly imperative to bring these forms of medicinal systems in front of the world so that cheap and affordable medicines could be propagated. The antioxidant studies on a number of sidha and Ayurvedic formulations and herbs were reported by us.[1-13] The present work is also a step in this direction in which the antioxidant studies of one herbo-minaral formulation Saraswatharishtam was undertaken. Saraswatharishtam consists of many plants among which Swagandha, Bramhi and Satavari, which are known to work as medhya rasayanas, i.e. medicines pertaining to brain and nerve related activities. These medicines also contain gold in a very small proportion. It is used to treat acute anxiety, fatigue, and insomnia, partial loss of memory, low grasping power, slurred speech and dementia and for certain neuro degenerative disease like Alzhemier disease and other cognitive diseases.[14]

The GC MS analysis and docking study results of Sarawatharishtam are already reported by us.[15, 16] Reactive Oxygen species is one the major cause of most of the diseases and one of the major mechanisms of action of medicines is their antioxidant activity. The present study involves in understanding the antioxidant activity of Saraswatharishtam by different assays. It was found that there is a perceptible antioxidant activity of these medicines as studied by various assay methods.

MATERIALS AND METHODS

Saraswatharishtam was procured from standard Ayurvedic vendor at Chennai. The formulation was processed according to standard procedures before subjecting to different antioxidant assays.

In vitro antioxidant Study

The objective was to do *In vitro* antioxidant activity studies of Saraswatarishtam by DPPH method, Hydrogen-peroxide scavenging activity, Total antioxidant activity by Ferric reducing (Ferric Thyocynate method), scavenging Hydroxyl radical scavenging activity and ABTS methods.

a. Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the Saraswatharishtam was examined on the basis of the scavenging effect on the stable DPPH free radical activity (Braca *et al.*, 2002).[17] Ethanolic solution of DPPH (0.05 mM) (300 l) was added to 40 l of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation (Yen and Duh, 1994).[18] Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100 Where AA and AB are the absorbance values of the test and of

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the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

Hydrogen Peroxide Scavenging Capacity

The ability of the Saraswatharishtam to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*, 1989).[19] A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both plant extracts and standard compounds were calculated.

Total Antioxidant Activity-Ferric Thiocyanate Method

The antioxidant activity of Saraswatharishtam and standards was determined according to the ferric thiocyanate method in linoleic acid emulsion (Mitsuda *et al*, 1996).[20] With this method peroxide formation occurred during the oxidation of linoleic acid oxidation. These compounds oxidized Fe^{2+} to Fe^{3+}. The latter ions form a complex with thiocyanate and this complex has a maximum absorbance at 500 nm.

Hydroxyl radical scavenging assay

Hydroxyl radicals were generated by a Fenton reaction (Fe^{3+}-ascorbate-EDTA-H_2O_2 system), and the scavenging capacity towards the hydroxyl radicals was measured by using deoxyribose method. The reaction mixture contained 2-deoxy-2-ribose (2.8 mM), phosphate buffer (0.1 mM, pH 7.4), ferric chloride (20 µM), EDTA (100 µM), hydrogen peroxide (500 µM), ascorbic acid (100 µM) and various concentrations (10-1000 µg/ml) of the test sample in a final volume of 1 ml. The mixture was incubated for 1 h at 37 °C. After the incubation an aliquot of the reaction mixture (0.8 ml) was added to 2.8% TCA solution (1.5 ml), followed by TBA solution (1% in 50 mM sodium hydroxide, 1 ml) and sodium dodecyl sulphate (0.2ml). The mixture was then heated (20 min at 90 °C) to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All experiments were performed in triplicates.

ABTS free radical scavenging assay

The antioxidant capacity of Saraswatharishtam was measured using 2, 2´-azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay (Re *et al*, 1999).[21] ABTS was dissolved in deionized water to 7 mM concentration, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12~16 h) in the dark before use. The resultant intensely-coloured ABTS•+ radical cation was diluted with 0.01 M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. The test compound was diluted 100 × with the ABTS solution to a total volume of 1 ml. Absorbance was measured spectrophotometrically at time intervals of 1 min after addition of each extract. The assay was performed at least in triplicate. Controls containing 990 µl of PBS, to replace ABTS, were used to measure absorbance of the extract themselves. The assay relies on the antioxidant capability of the samples to inhibit the oxidation of ABTS to ABTS•+ radical cation. The total antioxidant activities were expressed as mM trolox equivalent antioxidant capacity (TEAC).

The result of radical scavenging activities of Saraswatharishtam is expressed as percentage of inhibition which is calculated.

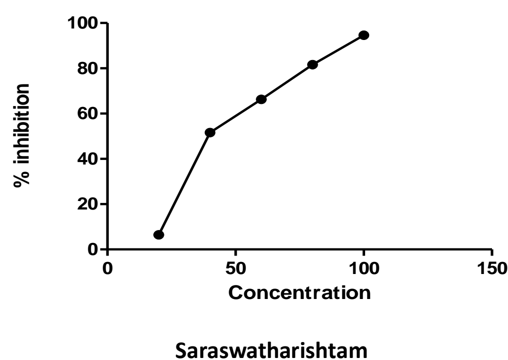
RESULTS AND DISCUSSION

The IC50 value of each method is mentioned hereunder. For DDPH method (42.84% for Ascorbic acid and 32.34% for Saraswatharishtam) (Table 1, Figure 1), for ABTS method BHT 39.0% and Saraswatharishtam 153.9% (Table 2, Figure 2), Hydrogen peroxide scavenging test BHT 26.06% and 69.57% for test drug (Table 3, Figure 3), Hydroxyl radical scavenging assay Ascorbic acid 60.5% and for Saraswatharishtam 162.7% (Table 4, Figure4), Ferric thyocynate method Ascorbic acid 55.99% and 126.6% for Sarawatharishtam (Table 5, Figure 5) was observed.

The in vitro antioxidant activity results also strongly indicate the antioxidant potential of Saraswatharishtam which augurs well with medicinal activity.

Table 1: In vitro antioxidant activity of Saraswatharishtam by DPPH Scavenging Activity

Sl.No.	% of Inhibition		
	Concentration (µg/ml)	Saraswatharishtam	Ascorbic Acid
1	20	6.43±2.86	38±3.6
2	40	14.9±4.12	51.6±3.4
3	60	34.2±2.57	66.31±1.72
4	80	48±3.26	81.62±2.46
5	100	52.72±4.63	94.6±1.63
	IC50	42.84	32.34



Saraswatharishtam

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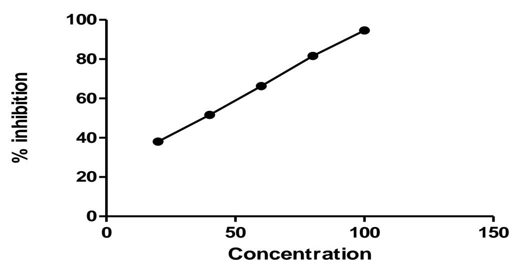
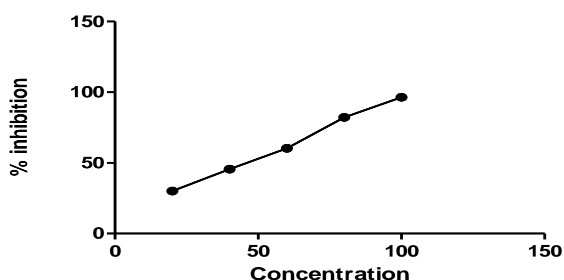


STANDARD (Ascorbic acid)

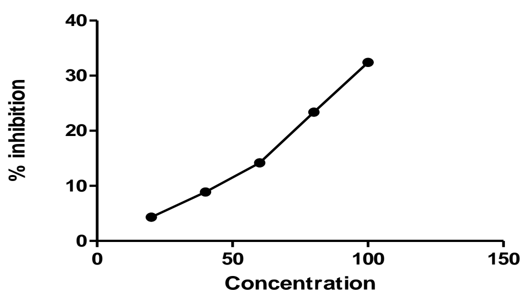
Figure 1: Shows the DDPH scavenging activity of Saraswatharishtam as compared to Ascorbic acid as standard.

Table 2: In vitro antioxidant activity of Saraswatharishtam by ABTS Scavenging Activity

S. No.	% of Inhibition		
	Concentration (µg/ml)	Saraswatharishtam	BHT
1	20	4.3±3.86	30±3.6
2	40	8.9±4.12	45.6±1.4
3	60	14.2±4.71	60.31±1.4 2
4	80	23.4±3.06	82.2±3.46
5	100	32.42±4.37	96.4±3.13
	IC50	153.9	39.0



Standard (BHT)

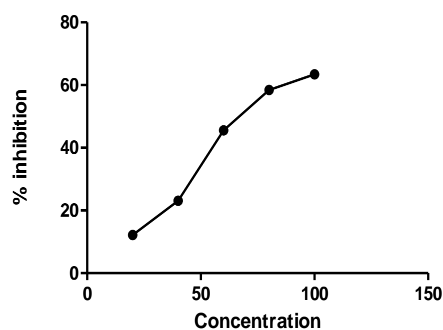


Saraswatharishtam

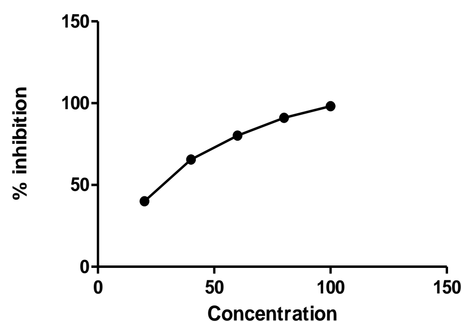
Figure 2: Graphs indicating In vitro antioxidant activity of Saraswatharishtam by ABTS Assay

Table 3: *In vitro* antioxidant activity of Saraswatharishtam by Hydrogen peroxide Scavenging Activity

Sl. No.	% of Inhibition		
	Concentration (µg/ml)	Saraswatharishtam	BHT
1	20	12.2±4.36	40±2.6
2	40	23.1±3.12	65.6±1.4
3	60	45.6±2.46	80.21±3.42
4	80	58.46±1.06	91±1.62
5	100	63.42±4.37	98.2±6.13
	IC50	69.57	26.06



Standard (BHT)



Saraswatharishtam

Figure 3: Graphs indicate in vitro Hydrogen peroxide Scavenging Activity of Saswatharishtam.

Table 4: In vitro antioxidant activity of Saraswatharishtam by Hydroxyl radical Scavenging Activity

S. No.	% of Inhibition		
	Concentration (µg/ml)	Saraswatharishtam	Ascorbic acid
1	20	8.3±3.86	12±6.6
2	40	12.3±2.16	25.6±4.2
3	60	18.2±3.21	45.31±2.12
4	80	24.62±2.16	68.2±4.26
5	100	36.32±1.64	84.4±2.61
	IC50	162.7	60.05

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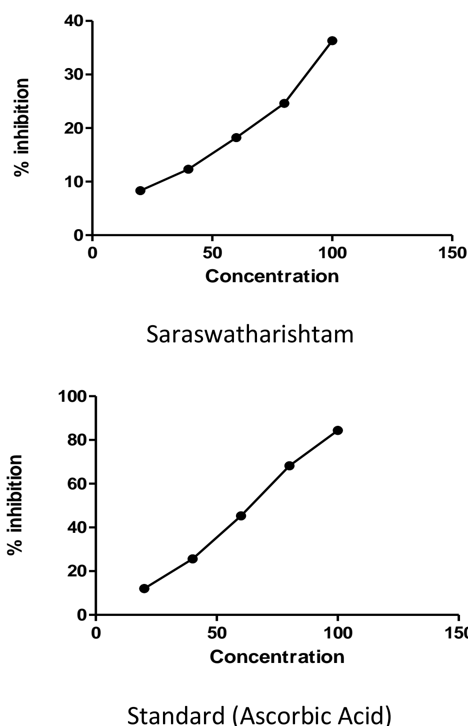


Saraswatharishtam

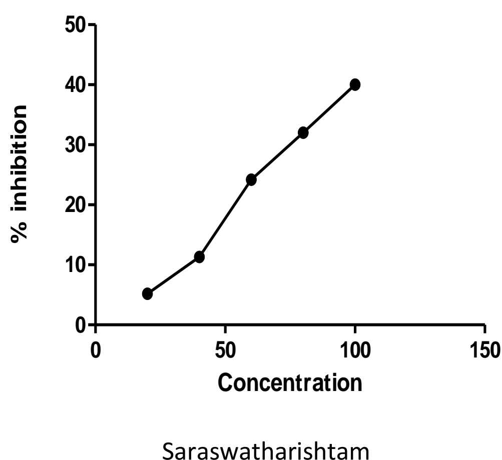
Standard (Ascorbic Acid)

Figure 4: Graphs indicate in vitro Hydroxyl radical Scavenging Activity of Saswatharistam.

In vitro Total antioxidant activity by ferric reducing activity

Table 5: In vitro antioxidant activity of Saraswatharishtam by Ferric thyocynate method.

Sl.No.	% of Inhibition		
	Concentration (µg/ml)	Saraswatharishtam	Ascorbic acid
1	20	5.2±3.86	14±8.6
2	40	11.3±5.16	25.6±6.2
3	60	24.2±1.21	46.31±3.7
4	80	32±3.16	88.2±3.26
5	100	40 ±4.64	94.4±5.61
	IC 50	126.6	55.99



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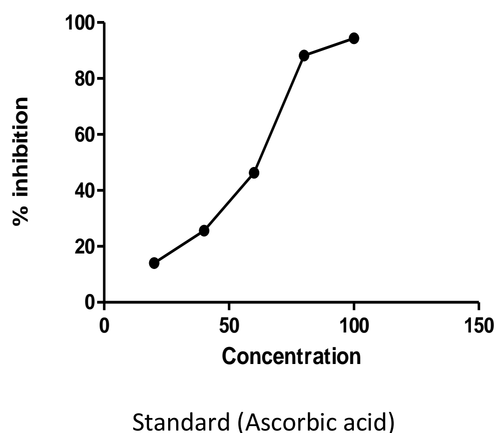

Standard (Ascorbic acid)

Figure 5: Graphs indicate In vitro Ferric thyocynate assay results of Saraswatharishtam.

CONCLUSION

From the above experiments it is clear that Sarawatharishtam has an excellent antioxidant activity which could be attributed to as one of the mechanisms for its activity as a potent medicine.

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