

## EVALUATION OF ANTI-OXIDANT ACTIVITY OF 'SUVARNA RAJ VANGESVARA'

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### ABSTRACT

*Suvarna Raj Vangeshwara* is a metallic drug preparation as per Ayurvedic scheduled text Rastarangini described as *Rasayana* having activity on various systems of body. In this study Antioxidant activity of *Suvarna raj vangesvara* was done by standard assays i.e. di-phenyl picryl hydrazyl radical scavenging assay, ferric chloride reducing power assay & lipid peroxidation assay. The low concentration of *suvarna raj vangesvara* showed very good anti-oxidant activity than that of butylated hydroxytoluene. In ferric chloride reducing assay, it was observed that the antioxidant activity of *suvarna raj vangesvara* is almost similar to that of standard i.e. Vitamin C. It also exhibited higher inhibitory activity on process of Lipid peroxidation in all concentrations as compared to the standard i.e. ascorbic acid. Thus, in all three assays *suvarna raj vangesvara* showed very good antioxidant activity and thus it is a potent tool for the remedy in Ayurveda to deal with the oxidative stress.

**Keywords:** *Suvarna Raj Vangeshwara*, Anti-oxidant, *Rasayana*, Di-phenyl picryl hydrazyl, Ferric chloride, Lipid peroxidation.

### INTRODUCTION

'*Suvarna Raj Vangeshwara*' is a metallic drug preparation described in an Ayurvedic scheduled text '*Rasataragini*'. The drug contains Mercury (Hg), Sulphur (S), Tin (Sn) and Ammonium Chloride (NH<sub>4</sub>Cl) (in the form of '*Navasagar*'). The drug belongs to a class of '*Kupipakva Rasayana*', which was produced through a specialized method. In this method the homogenous mixture of the ingredients is placed in a closed narrow mouthed long necked glass flask, wrapped in a multilayered clay smeared muslin cloth. The flask was then subjected to prolonged controlled heating in a sand bath. At the end of heating the product '*Kupipakva Rasayana*' in this study '*suvarna raj vangesvara*' was collected at the bottom of the flask. The flask was skillfully broken and the product '*suvarna raj vangesvara*' was collected and

stored for further analysis. Traditionally, '*Rasayana*' drugs are known to possess strong anti-oxidant activity. '*Rasayana*' plants with potent anti-oxidant activity have been reviewed for their traditional uses and mechanism of antioxidant action. Fifteen such plants have been dealt with in detail and some more plants with less work have also been reviewed briefly.<sup>1</sup> Seven plants (*Emblica officinalis* L., *Curcuma longa* L., *Mangifera indica* L., *Momordica charantia* L., *Santalum album* L., *Swertia chirata* Buch-Ham, *Withania somnifera* (L.) Dunal) contain antioxidant principles that can explain and justify their use in traditional medicine in the past as well as the present.<sup>2</sup> Antioxidant effect of active principles of *W. somnifera* may explain, at least in part, the reported anti-stress, immuno-modulatory, cognition-facilitating, anti-inflammatory and anti-aging effects

produced by them in experimental animals, and in clinical situations.<sup>3</sup> Thus it has been reported that the 'Rasayana' group of drugs possess antioxidant activity. However a major class of the drugs i.e. 'Rasaushadhi' remains unevaluated for the same context. References have been cited about 'Rasayana' properties of 'Rasaushadhis'. There is no such prominent study to assess this quality of these drugs till date.

As we are well aware about that reactive oxygen species are the key regulators in many diseases and antioxidants is the potent tool to scavenge these and thus minimize the complications of the disease like Cancer. Hence the present study aims at evaluation of *SUVARNA RAJ VANGESVARA* for its antioxidant activity in vitro, being a potent 'Rasayana' but unexplored drug in this context. In this study anti-oxidant activity of *suvarna raj vangesvara* was estimated in vitro by di-phenyl picryl hydrazyl radical scavenging assay, Ferric chloride reducing power assay and Lipid Peroxidation assay.

#### **MATERIALS AND METHODS:**

A sample of *Suvarnaraj wangeswar* was prepared in the department of 'Rasa-shastra and Bhaishjya kalapana' of R. A. Podar medical college, Mumbai as per the method prescribed by Sadanand Sharma in a classical Ayurvedic text 'Rasatarangini'.<sup>4</sup> Tin, Mercury, Sulfur, Ammonium chloride procured from the local market was used as raw materials for preparing *Suvarnaraj wangeswar*. These materials did not require any authentication; however Tin, Mercury, Sulfur and Ammonium Chloride were purified by using methods described in Ayurvedic classics. Tin was purified by melting it and pouring the molten metal in claimed lime water. Mercury was purified by grinding it with garlic and claimed lime thereafter washing it

with hot water, whereas sulfur was purified by melting it with ghee and then pouring the molten material in milk. The solidified sulfur gathered at the bottom of the milk was then washed with hot water. Ammonium chloride was dissolved in water and thereafter the solution was subjected to heating to evaporate the water. The dried Ammonium chloride was then collected and was used in preparation of 'Suvarna Raj Vangeswara'. All the purified material was then ground together to prepare a 'Kajjali', which was then filled in a specially prepared glass bottle covered with clay smeared muslin cloth. The bottle was then subjected to prolonged controlled heating in a sand bath. Heat ranges from 250<sup>0</sup> C to 600<sup>0</sup> C. It took around 6 hrs for active heating. After that apparatus was set to self cool. At the end of heating 'suvarna raj vangesvara' was collected at the bottom of the bottle. The bottle was skillfully broken and 'suvarna raj vangesvara' was collected and stored for further analysis. It was a golden brown scintillating product obtained as a mass which was further powdered and then used. 1/3<sup>rd</sup> amount of the kajjali was obtained again as final product, 'suvarna raj vangesvara'. The sample was subjected to screening of its antioxidant activity after in house standardization. A sample was sent to National chemical laboratory, Pune for analysis by XRD, being the most appropriate and sufficient test for standardization. Estimation of Antioxidant potential of *suvarna raj vangesvara* was assessed by performing three assays i.e. di-phenyl picryl hydrazyl assay, Ferric chloride reducing assay and Lipid peroxidation assay. These assays were performed by Haffkine institute, Mumbai.

1. Di-phenyl picryl hydrazyl radical scavenging assay:<sup>5,6</sup>

The effect of *Suvarnaraj wangeswar* on di-phenyl picryl hydrazyl radical was estimated using the gold standard method of di-phenyl picryl hydrazyl radical scavenging assay. di-phenyl picryl hydrazyl (0.0002%) (Sigma Aldrich) was freshly prepared in methanol. Butylated hydroxytoluene (5mg/ ml) was used as a standard. Sample was dissolved in aquaregia. The reaction mixture was prepared in

$$\% \text{ Inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

## 2. Ferric Chloride (FeCl<sub>3</sub>) reducing assay:<sup>7</sup>

Sample was prepared in concentration range of 10-70 µg/ml in phosphate buffered saline. Sample and standard was mixed with 2.5 ml of 1% potassium ferric cyanide. This reaction mixture was incubated at 50°C for 20 minutes. After incubation reaction mixture was cooled and 2.5 ml of 10% trichloroacetic acid was added followed by proper mixing. It was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of distilled water was added to 2.5 ml of the supernatant. To this reaction mixture 1ml of 0.1% ferric chloride was added followed by incubation of 10 mins at RT. Absorbance was measured at 700 nm. Ascorbic acid was used as a standard.

## RESULTS:

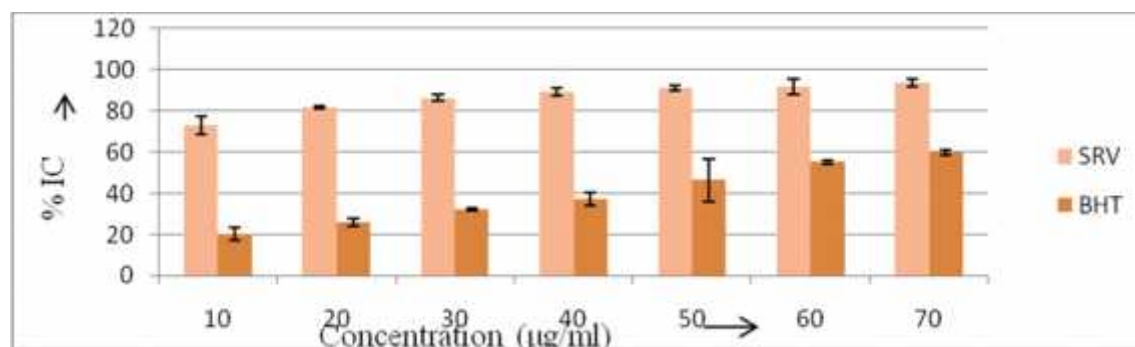


Fig. 1: DPPH radical scavenging assay of *Suvarnaraj vangeswar* and the Butylated hydroxytoluene.

the concentration range of 10-70 µg / ml in 200ul of methanol, to this 1800 µl of freshly prepared di-phenyl picryl hydrazyl was added. The reaction mixture was incubated in dark for 30 minutes and absorbance was read at 517 nm using UV spectrophotometer. The percentage inhibition was calculated according to formula of Yen and Duh (1994):

## 3. Lipid Peroxidation Assay:<sup>8,9</sup>

Reaction mixture was prepared by adding different concentrations (10-100 µg/ml) of sample in 0.5ml liver homogenate, 100µl of each 0.15 M Tris-HCL buffer, 15mM FeSO<sub>4</sub> and 6mM ascorbic acid. It was then incubated at 37°C for 1 hour. 0.01ml of SDS (9.8%) was added and centrifuged at 3000 rpm for 20 mins. Supernatant was then transferred in other test tubes. 1ml of thiobarbituric acid (0.8% w/v) was added to the supernatant. It was then incubated 20 mins at 90°C. On cooling, 2 ml of n-butanol was added. After the Centrifugation, absorbance of organic layer was measured spectrophotometrically at 532nm.

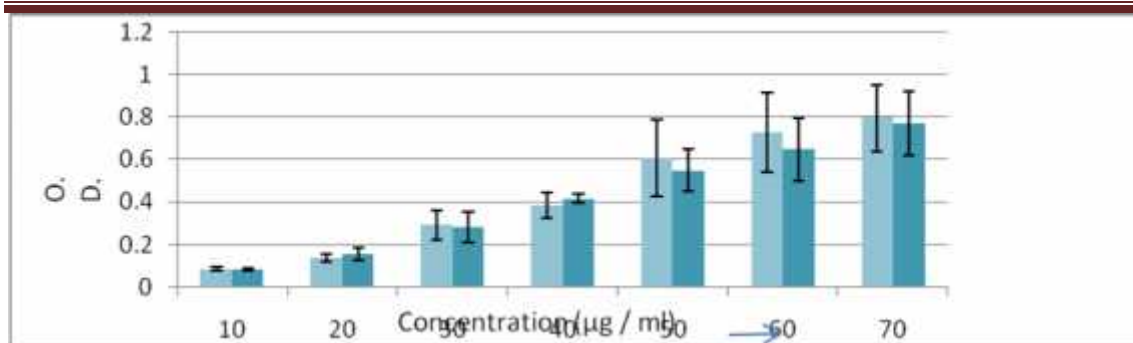


Fig. 2: Ferric Chloride (FeCl<sub>3</sub>) reducing assay of *Suvarnaraj vangeshwar* and Vitamin C.

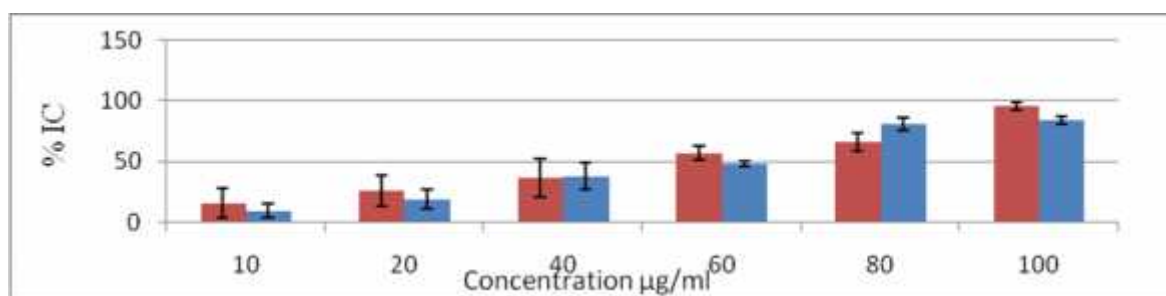


Fig. 3: Lipid Peroxidation assay of *Suvarnaraj vangeshwar* and Vitamin C.

The lowest concentration of *suvarna raj vangeshvara* 10 µg/ml showed IC value of 72% whereas that of butylated hydroxytoluene shows only 20%. *suvarna raj vangeshvara* 70µg/ml concentration showed IC value of 93% as compared to 59% by butylated hydroxytoluene. Thus, di-phenyl picryl hydrazyl radical scavenging activity of *suvarna raj vangeshvara* showed much higher antioxidant activity than that of butylated hydroxytoluene. [Fig.1] In ferric chloride reducing assay, it was observed that the antioxidant activity of *suvarna raj vangeshvara* is almost similar to that of standard i.e. Vitamin C at all concentrations i.e. from 10 µg/ml to 70µg/ml. [Fig.2] *suvarna raj vangeshvara* exhibits higher inhibitory activity on process of Lipid peroxidation in all concentrations as compared to the standard i.e. ascorbic acid. However the difference is more prominent in higher concentrations. 100 µg /ml *suvarna raj vangeshvara* showed IC value of 95 % as compared to 84% by Ascorbic acid at same concentrations. [Fig.3]

#### DISCUSSION AND CONCLUSION:

*Suvarnaraj wangeshwar* is basically a product of Tin. In house X-ray diffraction studies of *suvarna raj vangeshvara* have shown that it is a mixture of Sn, SnS and SnS<sub>2</sub>. However maximum peaks of

SnS<sub>2</sub> were observed. In di-phenyl picryl hydrazyl assay of *suvarna raj vangeshvara*, it showed dose dependent increase in antioxidant activity. Comparison of this activity with a standard antioxidant butylated hydroxytoluene indicates that the activity was much more prominent in *suvarna raj vangeshvara*. In Ferric chloride reducing power assay *suvarna raj vangeshvara* showed dose dependent increase in antioxidant activity almost similar to that of standard antioxidant i.e. Vit C. at all concentrations. *suvarna raj vangeshvara* exhibited higher inhibitory activity on process of Lipid peroxidation in all concentrations as compared to the standard antioxidant ascorbic acid. Thus, overall *suvarna raj vangeshvara* showed a potent and very good dose dependant antioxidant activity as compared the respective standards. *Suvarna Raj Vangeshwara* is found to possess a potent antioxidant activity through this study. It is used in *Ayurvedic* practice by *Ayurvedic* practitioners for multiple purpose may be because of its potent antioxidant activity since, reactive oxygen species plays important role in many pathological conditions and diseases. On this background, this multi-potential activity of this drug needs to be explored in future on *in-vitro* and *in-vivo* scales.

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