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B K Sarkar, K G Vasanthakumar, P Madhavikutty and V C Deep
National Research Institute for Panchakarma Central Council for Research in Ayurvedic Sciences Cheruthuruthy, Trissur, Kerala.

Correspondence
B K Sarkar
National Research Institute for Panchakarma Central Council for Research in Ayurvedic Sciences Cheruthuruthy, Trissur, Kerala.

Standardization and Antioxidant Evaluation of Varatika Bhasma

B K Sarkar, K G Vasanthakumar, P Madhavikutty and V C Deep

ABSTRACT

Current scenario lead to change in life style which has resulted in the increased production of free radicals which raise the oxidative stress and also play an important role in the immune system dysfunction. Hence there is need of a safe and effective antioxidant. In this regard Varatika Bhasm was screened for the free radical scavenging activity. Various antioxidant assay methods were used for this purpose such as hydroxyl radicals, super oxide radical scavenging activity and nitric oxide radical inhibition. Ascorbic acid (AA) was used as the standard antioxidant for the free radical scavenging assays. The study was carried out for different concentrations of drug in suspension form. The result of antioxidant activities shows significant scavenging of hydroxyl and super oxide radical with the minimum IC50 values of 54.8±0.53 mcg/ml and 91.21±0.22 mcg/ml respectively. Result of study suggested that Varatika Bhasm have excellent antioxidant activity against the different in vitro antioxidant models.

Keywords: Varatika bhasm , antioxidant, hydroxyl radicals, super oxide radical

1. INTRODUCTION

Free radicals are responsible for the diseases like Prameha (diabetes), Pandu (anemia), Vata vyadhi (neuro muscular diseases) etc1. An effective antioxidant can protect human body from these free radicals. Many herbals drugs and compound herbal preparations have been screened for their antioxidant properties but still there is a need for effective antioxidants. Varatika is categorised under Sadharana Rasavarga2 and also under Sudhavarga by Rasa scholars. Varatika is identified as the external shell of sea animal Cyprea moneta linn. It occurs in the coastal areas of the sea. Cypraea moneta, commonly known as the money cowry, because the shells were historically widely used in many Pacific and Indian Ocean countries as a form of exchange. Chemically, Varatika is identified as Carbonate of Calcium3. Dharana (amulet) of Varatika is practiced for the treatment of Balagraha (viral infections of children). Traditionally Varatika Bhasma used for the treatment of Agnimandya (Loss of appetite), Parinamasula (Duodenal ulcer), Grahani (Malabsorption syndrome), Rajayakshma (Tuberculosis), Karnasrava (Ottorhoea), Netraroga (Diseases of the eye) and Sukraksaya (Oligospermia). Varatika Bhasma was prepared by varatika or caracara which is yellowish tinge and has nodules on the back and oval in shape4. Since it can be effectively used for the treatment of ulcer which might be due to the oxidative stress; thus it was expected that Varatika Bhasma can act as potent antioxidant and by concerning this fact this work was conducted to establish direct antioxidant potential of Varatika Bhasma. Pharmacological Properties[5]are Rasa (Taste) – Katu (Pungent) Guna (Property) – Ushna (Hot) Virya (Potency) – Ushna (Hot) Vipaka (Post digestive effect) – Katu (Pungent)

2. MATERIALS AND METHODS

Varatika (Cowrie shells) were procured from local market. The drug was purified as per the methods mentioned in standard ayurvedic texts. Fresh Kulatha (Horse gram) was purchased from the market botanically identified as Macrotyloma uniflorum belonging to the family and its kashaya (decoction) was prepared for the purification process. Fresh Aloe vera was collected and its juice was used for making cakrikas or pellets to be used in the incineration process of Varatika. Chemical analysis was carried out employing modern sophisticated techniques such as FTIR, XRD and Scanning Electron Microscope (SEM).

2.1 Preparation of Varatika Bhasma

Varatika Bhasma was prepared by following standard method of ayurveda using Sodhana and Marana methods, other material were also used like; Vasa svarasa (Adhatoda vasica juice), nimbu svarasa (lemon juice), trikatu kashaya (decoction prepared with equal quantity of Piper longum, Piper nigrum and Zingiber officinale)[6-7.]

2.2 *In Vitro* Antioxidant Methods [8]

2.2.1 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of drug is directly related to its antioxidant activity. This method involves in vitro generation of hydroxyl radicals using Fe3+/ascorbate/ EDTA/ H2O2 system by Fenton reaction. The hydroxyl radicals formed by the oxidation are made to react with DMSO (dimethyl sulphoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow color with Nash reagent (2M ammonium acetate with 0.05M acetic acid and 0.02M acetyl acetone in distilled water), the intensity of which was measured at 412 nm spectrophotometrically against reagent blank. Scavenging activity was measured by using following formula:

(% scavenging) = 1-(Differences in absorbance of sample/Difference in absorbance of blank) X100

2.2.2 Superoxide scavenging

The superoxide anion radicals were generated in a mixture containing 0.5 ml of NBT (0.3mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml sample solution and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). The reaction was started by the addition of 0.5 ml PMS (Phenazine methosulphate) solution (0.12 mM) to the mixture, which was then incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample.

Scavenging activity was measured by using following formula:

Inhibition (%) = (absorbance of control – absorbance of test / absorbance of control) × 100

2.2.3 Nitric oxide radical scavenging

Sodium nitroprusside 5 mM was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of test sample, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ºC for 5 hours after which, 0.5 ml of Griess reagent was added. The absorbance of the chromophore was read at 546nm. Scavenging activity was measured by using following formula:

Inhibition (%) = (absorbance of control – absorbance of test / absorbance of control) × 100

3. RESULT AND DISCUSSION

Varatika, a mineral drug of animal origin is used in Ayurvedic therapeutics in many diseases. This preparation was analysed using sophisticated instruments like particle FTIR, SEM and XRD. A comparison of the FTIR spectra of the raw and final product indicates the changes occurred in the finger print region. SEM and XRD analysis further confirmed the occurrence of nano crystalline compounds in the final product (Figure 1).

Different concentrations of drug suspension were tested for their antioxidant properties in different in vitro models. The percentage of inhibition was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models (Table 1). The hydroxyl radical scavenging activity increased with increase in the concentrations of test compound. Superoxide free radicals were also scavenged with the increase in the concentration of test compound. The result of antioxidant activities shows significant scavenging of hydroxyl and super oxide radical with the minimum IC50 values of 54.8±0.53 mcg/ml and 91.21±0.22 mcg/ml respectively where ascorbic acid was used as standard. The percentage of inhibition in nitric oxide scavenging assay was maximum 84% at 500mcg/ml.

Table 1. *In vitro* Antioxidant assay

S. No.	*In vitro* Model	Concentration Used (mcg/ml)	IC50 (mcg)
1	Hydroxyl Scavenging Activity	100, 200, 300, 400, 500	54.8±0.53
2	Super Oxide Radical Assay		91.21±0.22
3	NO Scavenging Assay		74.3±0.23

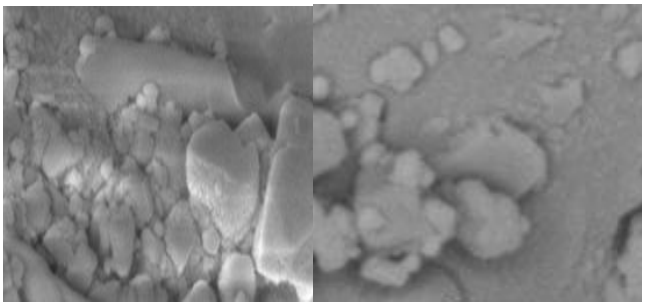

Figure 1.(a) SEM of raw Varatik (b) SEM of Varatika bhasma

4. CONCLUSION

In the preparation of Varatika Bhasma purifying agents play a vital role in removing the toxicity by forming complexes with organic compounds like flavones and tannins and also attains high therapeutic efficacy of these organic compounds (flavones and tannins) which are known to have potent antioxidant profile. The results of the present study indicate that Varatika Bhasma possess significant antioxidant activity when tested against different in vitro models. The antioxidant ability could be attributed to the phenolic compounds, especially flavonoids which possess antioxidant action. Thus Varatika Bhasma can be used for the prevention of free radical mediated diseases.

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