

EVALUATION OF ANTIOXIDANT ACTIVITY OF VANGESHWARA RASA

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ABSTRACT

Vangeshwara Rasa is a herbomineral formulation as per Ayurvedic scheduled text Rasendra Sara Sangraha containing Rasasindura and Vanga Bhasma. It is traditionally used as a prameha(Diabetes mellitus) and associated with metabolic dysfunction. The formulation contains possessing Rasayana(rejuvenative), Yogvahi(catalytic , efficacy enhancing) properties, which may help reduce oxidative stress associated with chronic diseases. Oxidative stress plays a significant role in the pathogenesis of various metabolic diseases due to excessive generation of reactive oxygen species(ROS). Vangeshwara Rasa demonstrated $21.01 \pm 1.86\%$ inhibition at $10 \mu\text{g/ml}$, which increased significantly to $52.69 \pm 1.45\%$ inhibition at $100 \mu\text{g/ml}$. The results indicate a concentration-dependent increase in free radical scavenging activity.

KEYWORDS: Vangeshwara Rasa, Antioxidant, DPPH assay, Free radical scavenging (ROS).

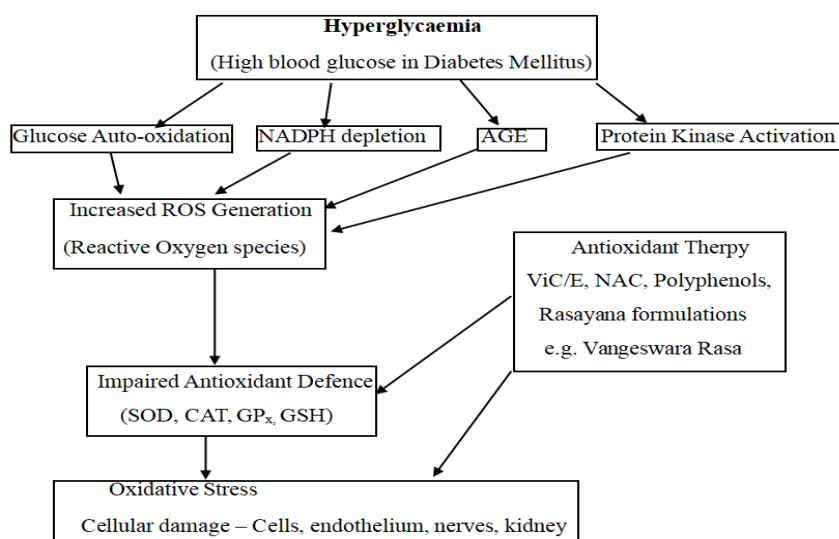
INTRODUCTION

Ayurveda, the classical Indian system of medicine, it has a large no. of herbomineral preparations known as Rasaushadhis, which have potent has low dosage and rapid therapeutic action. Vangeshwara rasa is a classical herbomineral preparation consisting mainly of Rasasindura and Vanga Bhasma. It has been widely used in Ayurvedic therapeutics, particularly in prameha.[1] Traditionally, 'Rasayana' drugs are known to possess strong anti-oxidant activity. References have been cited about Rasayana properties of 'Rasaushadhis'. Vanga Bhasma is well known for its Pramehaghna(antidiabetic), Rasayana(rejuvenative), Balya(strength-promoting), and mutrala(diuretic) properties. Rasasindura is a Kupipakwa Rasayana formulation, it is known for its Rasayana, Yogvahi, Deepana, Balya, and Ojovardhaka properties.

Rasasindura acts as rejuvenative (Rasayana) and aphrodisiac (Vrishya). It nourishes the body, improves vitality and immunity, increases strength and longevity and helps maintain good health. It is considered useful in promoting overall well-being.

Since oxidative stress is implicated in the progression of metabolic diseases, evaluation of the antioxidant potential of Vangeshwara Rasa is of scientific importance. Oxidative stress is caused by an imbalance between free radical generation and antioxidant defense mechanism. Excessive production of ROS(Reactive oxygen species) leads to cellular damage and contributes to various pathological including diabetes mellitus, cardiovascular disorder and aging. Antioxidant neutralise free radical and prevent oxidative damage. Despite its extensive traditional use, scientific documentation of antioxidant potential of Vangeshwara Rasa remains limited. The present study was therefore undertaken to evaluate in-vitro free radical scavenging activity using DPPH assay, a widely accepted, rapid and reproducible method for the preliminary assessment of antioxidant capacity.[2]

Oxidative stress in Diabetes Mellitus & Impaired Antioxidant Defence in Diabetes



Aim: To evaluate the antioxidant activity of Vangeshwara Rasa by the DPPH radical scavenging assay.

Objectives:

- To determine the free radical scavenging activity of Vangeshwara Rasa.
- To compare its antioxidant activity with standard Ascorbic acid.
- To evaluate the concentration-dependent antioxidant effect.

MATERIALS AND METHODS:

A sample of Vangeshwara Rasa was prepared in the department of Shri Narayan Prasad Awasthi Govt. Ayurved college Raipur C.G., as per the method prescribed in a classical text Rasendra Sara Sangraha. Vanga(Tin),[1] Hingula, Gandhaka,procured from the local market was used as raw materials for preparing Vangeshwara Rasa. These materials did not require any authentication, however Tin ,Hingula, Gandhaka were purified by using methods describe in Ayurvedic classics. Vanga(Tin) was purified by melting it and pouring the molten metal in Til Taila, Takra, Kanji. Gomutra, Kulattha Kwatha for Samanya Shodhana and after that for Vishesh Shodhana pouring the molten metal in mixture of Nirgundi Swarasa and Haridra Churna.[3]

Purified Vanga(Tin) was melted in an iron pan and subjected to Jarana using apamarga panchanga until it was converted into a fine powder. The Jarita Vanga was triturated with the kumari swarasa as Bhavana dravya, pellets were prepared, and Puta was administered according to classical guidelines. Repeated cycles of Bhavana and Puta were carried out until a soft, fine, lustreless Vanga Bhasma satisfying the classical Bhasma Pariksha(Varitara, Rekhapurnatva, and Nischandratva) was obtained.[4]

Hingula was purified by Meshi Ksheera and Nimbu swarasa. Purified Hingula was finely powdered and subjected to the classical extraction procedure using the Urdhvapatana Yantra. On controlled heating, Mercury vapours were generated and condensed in the upper chamber to obtain purified Hingulottha Parada. The collected mercury was washed thoroughly with warm water and dried before further pharmaceutical processing.[5]

Gandhaka was purified by melting it with ghee and then pouring the molten material in milk. The solidified Gandhaka gathered at the bottom of the milk was then washed with hot water.[6]

The extracted Hingulottha Parada was triturated with purified Gandhaka in equal portion to prepare Kajjali. Trituration was continued until a fine, smooth, lustreless black powder exhibiting the classical characteristics of Siddha Kajjali was obtained. The prepared Kajjali was filled into a Kacha Kupi, sealed with multiple layers of clay-smeared cloth(Kapadmitti), and subjected to Kramagni in a Valuka Yantra. After completion of the heating schedule and self-cooling, the bottle was carefully broken, and Rasasindura deposited in the neck of the bottle was collected and preserved in an airtight container.[7]

The prepared Rasasindura and Vanga Bhasma were mixed in the prescribed portion and triturated thoroughly in a Khalva Yantra. The mixture was levigated with the honey until a homogenous mass suitable for Vati preparation was obtained , uniforms Vati were prepared, shade dried, and stored in airtight glass containers. The final formulation was subjected to organoleptic and physicochemical evaluation before analytical and pharmacological studies.[8]

The sample was subjected to screening of its antioxidant activity after in house standardization. A sample was sent to Ravishankar shukla university Raipur C.G., for estimation of Antioxidant potential of Vangeshwara Rasa was assessed by performing DPPH assay. These assay were performed by Ravishankar shukla university Raipur C.G..

DPPH (Di phenyl picryl hydrazyl radical scavenging assay) – The effect of Vangeshwara Rasa on di-phenyl picryl hydrazyl radical was estimated using this widely accepted method of phenyl picryl hydrazyl radical scavenging assay. Di phenyl picryl hydrazyl(0.0002%) was freshly prepared in methanol. Ascorbic acid(5mg/ml) was used as standard. Sample was dissolved in aqua regia. The reaction mixture was prepared in the concentration range of 10-100µg/ml in 200 ul of methanol, to this 1800µl of freshly prepared di phenyl picryl hydrazyl was added. The reaction mixture was

incubated in dark for 30 minutes and absorbance was read at 517nm using UV spectrophotometer. The percentage inhibition was calculated according to formula of Yen and Duh(1994).[8][9]

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

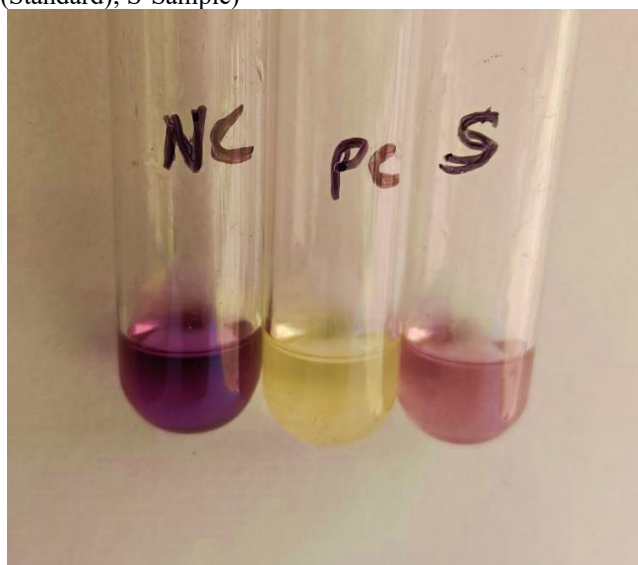
RESULTS:

Ascorbic acid produced $63.78 \pm 0.53\%$ inhibition at $10 \mu\text{g}$. Vangeshwara Rasa produced $21.01 \pm 1.86\%$ inhibition at $10 \mu\text{g}$ and $52.69 \pm 1.45\%$ inhibition at $100 \mu\text{g}$, demonstrating a clear concentration-dependent increase in radical scavenging activity.

DPPH ACTIVITY

Samples	DPPH Radical Scavenging Activity (%) (Mean $\pm$ SE)
AA $10 \mu\text{g}$	63.78 ± 0.53
S $10 \mu\text{g}$	21.01 ± 1.86
S $100 \mu\text{g}$	52.69 ± 1.45

(NOTE: AA- Ascorbic Acid (Standard); S-Sample)



DISCUSSION:

The DPPH assay is a well established, a sensitive and economical method for evaluating the free radical scavenging capacity of natural products. It is based on the ability of an antioxidant to donate a hydrogen atom or electron to the stable DPPH radical hydrazine with a measurable decrease in absorbance at 517nm.

In the present study, Vangeshwara Rasa demonstrated a clear concentration dependent scavenging of the DPPH radical, confirming its ability to neutralise free radical in-vitro. Although its potency was lower than that of the pure standard ascorbic acid- an expected finding, since a complex multi component formulation is unlikely to match the activity of a purified vitamin – the observed activity is nonetheless pharmacologically meaningful.

The antioxidant potential of the sample was evaluated using the DPPH radical scavenging assay, which is widely accepted method for determining the free radical scavenging capacity of plant extracts and herbal formulations. A higher percentage of DPPH inhibition indicates stronger antioxidant activity. In the present study, ascorbic acid(AA, $10\mu\text{g}$), used as the standard antioxidant, exhibited $63.78 \pm 0.53\%$ DPPH radical scavenging activity. The test sample showed $21.01 \pm 1.45\%$ inhibition at $10 \mu\text{g}$, demonstrating a concentration-dependent increase in antioxidant activity.

The increase in DPPH radical scavenging activity with increasing concentration suggests that the sample contains bioactive compounds capable of donating hydrogen atoms or electrons to neutralize free radicals. Although the antioxidant activity of the sample at $100 \mu\text{g}$ was lower than that of the standard ascorbic acid, it exhibited considerable free radical scavenging potential, indicating the presence of natural antioxidant constituents. The colour change observed from deep violet to pale yellow/pink in the reaction mixture further confirms the reduction of DPPH radicals by the antioxidant compounds present in the sample. Such antioxidant activity may be attributed to phytochemicals such as phenolic compounds, flavonoids, tannins, or other reducing constituents, which are known to protect against oxidative stress.

The antioxidant activity of Vangeshwara Rasa may be attributed to several factors. The bhasmas incorporated in the formulation contribute biologically relevant trace elements, and the repeated incineration involved in their preparation yields particles of extremely small size with a high surface area to volume ratio, which may enhance their electron donating and radical quenching capacity. In addition the organic phytoconstituents introduced during bhavana (levigation)

with herbal juices and decoctions) are likely to contribute polyphenolic and other reducing components that enhance the overall antioxidant effect.

Overall, the findings indicate that the test sample possesses significant antioxidant activity in a dose-dependent manner and may serve as a potential natural source of antioxidants. However, further studies involving phytochemical characterization, isolation of active constituents, and additional in vitro and in vivo antioxidant assays are required to validate these findings and establish its therapeutic significance.

CONCLUSION: The present study demonstrated that the test sample possesses significant antioxidant activity as determined by the DPPH radical scavenging assay. The antioxidant effect increased with increasing concentration, indicating a dose-dependent response. Although the activity of the sample was lower than that of the standard antioxidant (ascorbic acid), the sample exhibited appreciable free radical scavenging potential, particularly at 100 µg. These findings suggest that the sample contains bioactive antioxidant constituents that may help reduce oxidative stress. Since oxidative stress is central to the pathogenesis and progression of diabetes mellitus and its complications. They also lend objective substance to the Rasayana property classically attributed to Rasaushadhis, The results should be interpreted within the limits of a single in vitro assay performed at two concentrations. Such investigation would help establish Vangeshwara Rasa as a scientifically validated antioxidant adjuvant in the management of Prameha and allied metabolic disorders. Further phytochemical analysis and in vitro/in vivo studies are recommended to identify the active compounds and confirm its therapeutic potential.

REFERENCES

1. Sharma S, editor. Rasendra Sara Sangraha. Varanasi: Chaukhambha Orientalia; 2012.
2. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;181(4617):1199-1200.
3. Kulkarni DA, editor. Rasa Ratna samucchaya. New Delhi: Meharchand Lalchhmandas Publications; 2020. Chapter 5 P no. 124.
4. Ayurvedic Formulary of India. Part 1. 2nd ed. New Delhi: Ministry of Ayush, Government of India; 2003.
5. Vaidya Jadavji Tricumji Acharya, editor. Ayurveda Prakash. Bombay: Bora Bazar Street, Fort; 1913 A D. Chapter 3 P no 75.
6. Tripathi I, editor. Rasendra Sara Sangraha. Varanasi: Chaukhambha Orientalia; Reprint edition.
7. Shastrina kashinath pandit, editor. Rasa Tarangini. New Delhi: Motilal Banarasidas publishing House; 2021. Chapter 2 P no 16.
8. Yen GC, Duh PD. Scavenging effect of methanolic extracts of peanut hulls on free radical and active -oxygen species. *J Agric Food Chem*. 1994;42(3):629-632.
9. Naik AA, Kulkarni AM, Ankulkar PV, Savrikar SS, Chowdhary AS. Evaluation of Anti-oxidant activity of Suvarnraj Vangeshwara. *Int Ayurvedic Med J*. 2013;1(6):98-102.
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