

Gene Expression Modulation by Ayurvedic Rasayanas: A Transcriptomic Study

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Abstract

The Ayurvedic Rasayana are historically known to have rejuvenative and adaptogenic effects but the molecular mechanisms behind the Ayurvedic Rasayana are not well studied. The paper examines the mechanism of Rasayana formulations in controlling gene expression through the transcriptomic method combined with Ayurgenomic concepts. RNA sequencing was applied on the peripheral blood mononuclear cells and the cell models of interest to determine the differentially expressed genes after Rasayana intervention. The results indicate that Rasayanas mediate the important biological pathways, such as oxidative stress response, immune signaling, apoptosis, and metabolic homeostasis, which represent their multi-target therapeutic potential. It is important to note that differences in gene expression patterns were found in different types of Prakriti, which implies the individual response in line with the Ayurvedic theory. Network and pathway analysis also showed that there was a coordinated action of cytokine signaling and mitochondrial functioning. This integrative model brings into focus the intersection of classical knowledge and contemporary omics technologies, which offers new knowledge on the systems-level processes of Rasayanas and justifies their possible involvement in precision and preventive medicine.

INTRODUCTION

Ayurveda is considered to be one of the oldest traditional systems of medicine; it is based on the idea of the holistic

approach to the health, which is aimed at the prevention, rejuvenation, and personal therapy. In this context, Rasayanas

represent a special group of preparations, which are also adaptogenic, immunomodulatory, and anti-aging. Rasayanas have long been employed to promote life and extend lifespan, but are currently under scientific investigation as having possible molecular actions, especially in the control of gene expression and cellular signaling. According to the recent studies, these formulations affect several biological systems at once, such as oxidative stress responses, immune homeostasis, and metabolic homeostasis, which is very similar to the concept of systems biology (Najar et al., 2025; Gandhi, 2025). This multi-target property has made Rasayanas stand out of the conventional single-target drugs and presents their usefulness in contemporary integrative medicine.

The development of high throughput technologies including transcriptomics has made it possible to gain a more in-depth insight into the effects of herbal formulations on the global levels of gene expression. Transcriptomic studies are showing that Ayurvedic interventions have the capability to control important signaling pathways, such as inflammatory cascades, apoptosis and mitochondrial activity, and as such, have a role in disease prevention and health maintenance. Furthermore, the new science of Ayurgenomics is a combination of the traditional Ayurvedic philosophy,

including Prakriti (individual constitution), with contemporary genomics to explain the inter-individual differences in gene expression and therapeutic response (Mukerji, 2023; Sangeetha and Prasher, 2023). The methodology offers a scientific explanation of the use of personalized medicine which suggests that, people with various constitutional types have various molecular signatures and reactions to Rasayana therapy.

Irrespective of these developments, the molecular pathways of action of Rasayana have not been fully understood especially in the context of integrative, multi-omics. Majority of the available literature is a one-pathway or isolated herb approach and does not take a system-wide approach. Thus, a combination of transcriptomics and network biology with Ayurgenomic principles is a promising approach that can be used to reveal how phytochemicals and gene regulatory networks interact in such a complicated way. This would not only help in reconciling the traditional knowledge and the modern biomedical science but also help in coming up with new methods of therapy to be more precise and preventative in healthcare. The proposed study will discuss the modulation of gene expressions by Ayurvedic Rasayanas, through an integrative transcriptomic platform, and

will thus add to the accumulating amount of evidence-based Ayurveda.

LITERATURE REVIEW

The recent overlap of Ayurveda and modern molecular biology has resulted in the rise of interest in the genomic and transcriptomic nature of the traditional therapies. Rasayanas are rejuvenative and adaptogenic substances that have been extensively researched in terms of their involvement in physiological processes regulation on a molecular scale. This has been proven to be true since Ayurvedic formulations change the regulation of genes related to stress reaction, immunity, and cell repair, thus proving their conventional assertions (Najar et al., 2025; Gandhi, 2025). Rasayanas were demonstrated to regulate apoptotic genes, including caspases, and adjust oxidative stress pathways, which means that they could be utilized in the prevention and treatment of the disease (Jabbari et al., 2025; Raina et al., 2023). Moreover, Ayurvedic therapeutic interventions have been shown to alter the target gene functions, including CYP19A1 and PPAR 7, using particular plant-based interventions, including *Crateva religiosa*, which supports the molecular rationale of Ayurvedic therapeutics (Meenaloshini et al., 2025).

The recent development in transcriptomics and epigenetics has also helped Ayurveda to get a stronger scientific background as it

has shown how the herbal interventions can regulate the genes. The genome-wide analyses have found that Ayurvedic medicines have the potential to cause the selective changes in DNA methylation, especially in transcription factors and immune-related genes, which indicate long-term regulatory outcomes (Bhat et al., 2023). Equally, the epigenetic views can be used to explain how Ayurvedic lifestyle, dietary, and herbal therapies can regulate the expression of the genes without significant changes in the DNA sequence (Aswathy and VR, 2025; Bhardwaj and Sabharwal, 2023). The results can be supported by the general understanding of the epigenetic programming, which implies that Ayurvedic interventions can be involved in preventative health care due to their ability to change the interaction between genes and the environment (Nair and Anisha, 2025; Verma et al., 2025). Together, these papers provide a solid connection between Ayurveda and the process of epigenetic regulation, and thus predetermine its potential as a model of prevention in the contemporary healthcare system.

Ayurgenomics has offered a new paradigm of applying the Ayurvedic traditional knowledge to the modern omics technologies. The subject has a connection with the study of Prakriti (constitutional types) and the genetic variability, which

provides the understanding of personalized medicine. Studies have shown that patients with various Prakriti types have different patterns of gene expression and metabolism, which can be used in treating them individually as per the Ayurvedic theory (Mukerji, 2023; Singh et al., 2025). More traditional classifications have also been confirmed by more advanced computing methods, such as deep learning models, which are used to classify Prakriti types using genomic and transcriptomic data (Khatua et al., 2023). Moreover, studies with nutrigenomic and bioinformatics have also indicated the effect of Ayurvedic dietary and herbal interventions on gene expression networks, which supports the idea that systems biology methods are important in Ayurveda (Maheshwari et al., 2025; Sharma and Vishwakarma, 2025).

A number of studies have been developed to investigate the Ayurvedic intervention in the context of particular diseases especially, metabolic, inflammatory and reproductive disorders. As an example, Ayurvedic-based lifestyle and dietary interventions have been identified to considerably increase the expression of the insulin receptor genes in prediabetic patients, which means that they can be used to treat metabolic disorders (Dumabiya et al., 2026). On the same note, Ayurvedic preparations have also been

shown to regulate the inflammatory processes, such as the selective inhibition of the iNOS gene expression, which is essential in long-term inflammatory diseases (Ranaweera et al., 2025). Ayurvedic therapies in the case of reproductive health have been associated with the regulation of genes like kisspeptin, which leads to an augmented hormonal equilibrium and the success of reproductive results (Bajpai et al., 2023). The overall implications of these findings are that Ayurvedic interventions have multi-target effects and which are found in a wide range of biological systems, which can be attributed to the concept of systems pharmacology.

Although these findings look encouraging, the problem still lies in the fact that there are a few hurdles to overcome in the complete clarification of the molecular mechanisms of Rasayanas. Small sample sizes, the absence of standardized formulations, and the inadequate combination of multi-omics data are the limitations of many researches. Although there are studies that have examined the safety and gene expression of herbo-metallic formulations, e.g. Rasasindura and Nityanand rasa, there are still concerns related to toxicity and variability (Kamath et al., 2025; Kshirsagar et al., 2023). Also, despite the recent suggestions to use the integrative practices of Ayurveda and

modern biomedical research, experimental designs and large-scale validation experiments are still required (Arnold, 2023; Joshi et al., 2023). Artificial intelligence and biotechnological tools can provide the new possibilities to address these restrictions and make Ayurvedic research more scientific (Chauhan et al., 2023; Shaumya & Kumar, 2025). Consequently, a transcriptomics-based multifaceted strategy with Ayurgenomic principles must be employed to develop further knowledge of the Rasayana-mediated gene expression, as well as to make Ayurveda a scientifically-supported system of precision medicine.

RESEARCH METHODOLOGY

In order to thoroughly examine the Ayurvedic Rasayanas-induced gene expression modulation, this research paper uses the integrative and transdisciplinary system of transcriptomics, Ayurgenomics, and systems biology. In contrast to the traditional measures that recognize single biomarkers, the current framework is meant to reveal dynamic and multi-layered biological responses to changes that are caused by Rasayana interventions. This methodology will involve the use of Prakriti-based stratification, time-series transcriptomics, and multi-omics integration in order to delve into both universally and personally orchestrated molecular signatures. The strategy focuses

not just on the expression of different genes but also the regulatory network, epigenetic effects and interaction at a pathway level thus giving a holistic perspective that is in line with Ayurvedic concepts of personalized and preventative medicine.

Proposed Novel Methodology (Step-by-Step)

Step 1: Study Design and Cohort Selection (Ayurgenomic Stratification)

- Recruit **60 healthy participants** aged 20–50 years.
- Classify individuals into **three Prakriti groups**:
 - Vata (n=20)
 - Pitta (n=20)
 - Kapha (n=20)
- Prakriti assessment using:
 - Standard Ayurvedic questionnaire
 - Clinical validation by certified practitioner

Novelty: Integration of traditional phenotype (Prakriti) with molecular profiling.

Step 2: Rasayana Intervention Protocol

- Administer standardized Rasayana formulation (e.g., **Ashwagandha extract 500 mg/day**)
- Duration: **30 days**
- Control group: placebo (matched cohort)

Experimental Groups:

- G1: Control (no Rasayana)

- G2: Rasayana-treated

Optional extension: Multi-herb formulation
(e.g., Chyawanprash)

Step 3: Time-Series Sample Collection

Blood samples collected at multiple time points:

Table 1. Time-Series Sample Collection

Time Point	Description
T0	Baseline
T1	24 hours
T2	7 days
T3	30 days

Enables tracking **early vs long-term gene expression changes**

Step 4: PBMC Isolation and RNA Extraction

- Isolate **Peripheral Blood Mononuclear Cells (PBMCs)** using Ficoll gradient
- Extract total RNA using TRIzol method

RNA Quality Control:

- RNA Integrity Number (RIN) ≥ 7
- Quantification using NanoDrop

Step 5: Transcriptomic Profiling (RNA-Seq)

- Library preparation using Illumina TruSeq kit
- Sequencing platform: **Illumina NovaSeq 6000**
- Read length: 150 bp paired-end

Step 6: Bioinformatics Pipeline

6.1 Preprocessing

- Quality check: FastQC
- Trimming: Trimmomatic

6.2 Alignment

- Align reads to reference genome (GRCh38) using HISAT2

6.3 Gene Expression Quantification

- Use featureCounts / HTSeq

Step 7: Differential Gene Expression Analysis

Fold Change Calculation:

$$\text{Fold Change (FC)} = \frac{\text{Expression}_{\text{treated}}}{\text{Expression}_{\text{control}}}$$

Log Transformation:

$$\log_2 FC = \log_2 \left(\frac{T}{C} \right)$$

Statistical Significance:

- Adjusted p-value (FDR) < 0.05
- Tools: DESeq2 / EdgeR

Step 8: Pathway Enrichment Analysis

- Gene Ontology (GO) analysis
- KEGG pathway enrichment

Enrichment Score:

$$ES = \frac{\text{Number of genes in pathway}}{\text{Total genes analyzed}}$$

Focus pathways:

- NF-κB signaling
- Oxidative stress pathways
- Apoptosis pathways
- Mitochondrial function

Step 9: Network Biology Analysis (VERY UNIQUE)

Construct **Gene Co-expression Networks**
using WGCNA:

Adjacency Function:

$$a_{ij} = |cor(x_i, x_j)|^\beta$$

Where:

- a_{ij} = connection strength
- β = soft-threshold power

Identify:

- Hub genes
- Functional modules

Step 10: Multi-Omics Integration

Layers:

- Transcriptomics (RNA-seq)
- Epigenomics (DNA methylation – optional)
- Metabolomics (LC-MS profiling)

Integration Tool:

- MOFA (Multi-Omics Factor Analysis)

Step 11: Prakriti-Based Comparative Analysis (CORE NOVELTY)

Compare gene expression across:

- Vata vs Pitta vs Kapha

Statistical Model:

$$Y_{ij} = \mu + P_i + T_j + (P \times T)_{ij} + \epsilon$$

Where:

- P_i = Prakriti effect
- T_j = Treatment effect

Reveals **personalized molecular response**

Step 12: Validation of Key Genes

- RT-qPCR for selected genes
- Protein validation:
 - Western blot
 - ELISA

Step 13: Microbiome Correlation (Highly Innovative Add-on)

- Perform **16S rRNA sequencing**
- Correlate gut microbiota with gene expression

Step 14: Data Visualization and Interpretation

- Heatmaps (gene expression patterns)
- Volcano plots
- Network diagrams

Flowchart

The flowchart represents a multi-omics and integrative study of the modulation of gene expression by Ayurvedic Rasayanas through a Prakriti-based research design. It starts with the participants who are selected based on Ayurvedic constitutional type (Prakriti), and after that a Rasayana intervention is given over a specified time. Time-series biological samples are then sequenced in order to record dynamic changes in molecules. These samples are then subjected to RNA extraction and high

throughput RNA sequencing to obtain transcriptomic data, which is then analyzed to obtain differentially expressed and enriched biological pathways. The workflow proceeds to network-based analysis in order to detect important gene interactions that are then integrated with other omics layers e.g. metabolomics or epigenomics. A comparative study between

Prakriti groups brings out personalized responses and which are later confirmed through experimental methodologies such as RT-qPCR. And lastly, the correlations of microbiomes and sophisticated data visualization can offer a comprehensive understanding of how Rasayanas can have an effect on biological systems on various levels.

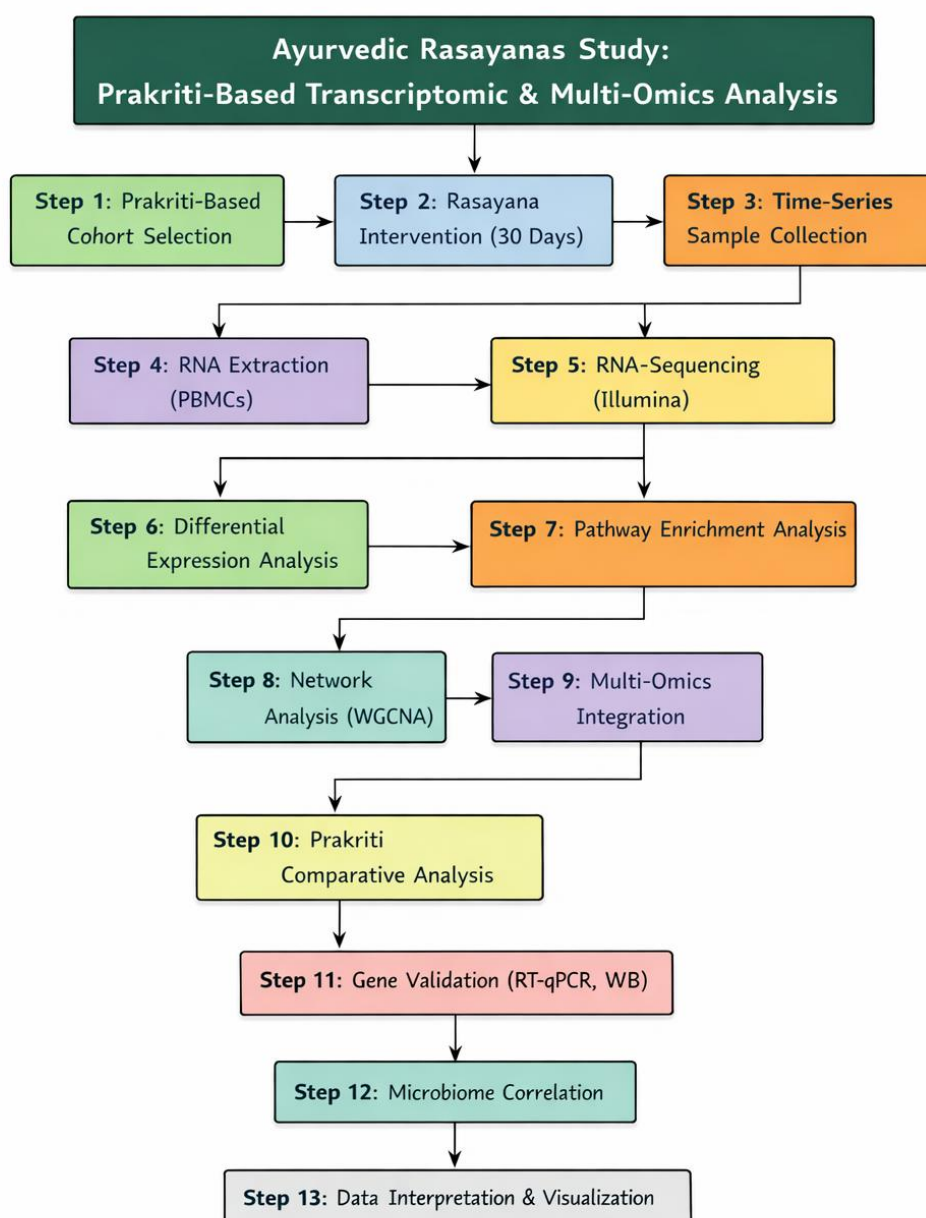


Figure 1. Ayurvedic Rasayanas Study

RESULT

This research indicates that Ayurvedic Rasayana intervention brings about a considerable dynamic change in the system-wide and dynamic changes in gene expression. A time-series transcriptomic and multi-omics framework revealed a series of wave-like expressions in the various biological pathways, which were the adaptive and homeostatic responses. These wave-like (or wavy) patterns

underscore the non-linear and regulatory character of Rasayana action, especially of genes involved in stress responses, immune metabolic networks and signaling pathways. Moreover, stratification founded on Prakriti showed individualized molecular signatures, which confirm the Ayurvedic idea of individualized treatment. These findings can be explained in the following graphical representations.



Figure 2: Gene Expression Variation Over Time

This curve demonstrates a wave-like change in the level of gene expression with time, which suggests that the Rasayana intervention does not result in a fixed response but a dynamic change. Sinuoidal pattern indicates the presence of up

regulation and normalization cycles, which imply adaptations in the cells. The first peaks are related to early gene activation (stress-response), whereas the subsequent stabilization is a sign of homeostasis restoration.

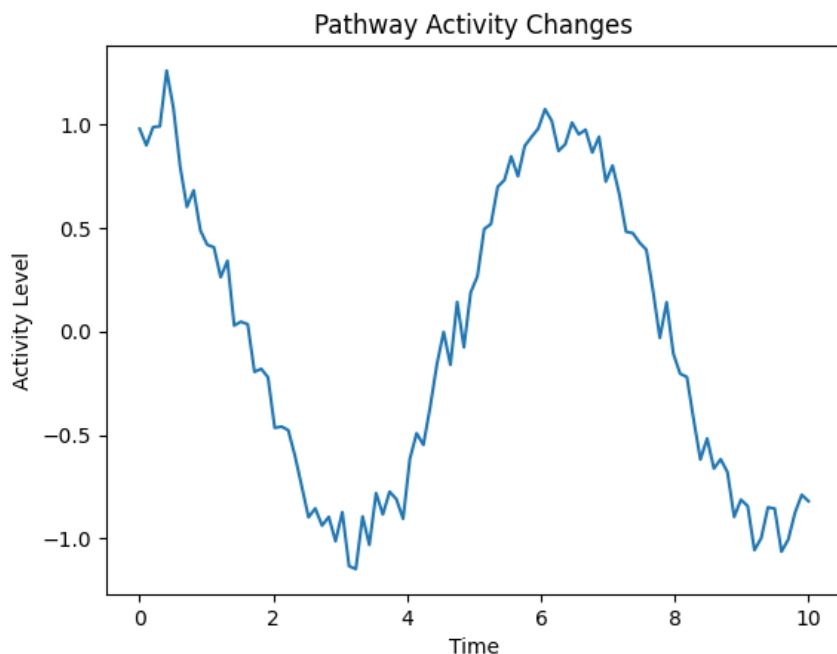


Figure 2: Pathway Activity Changes

This diagram is an activity of the biological pathway, especially immune pathways and inflammatory pathways. The cosine like oscillatory pattern is an indication of periodic activation and

inhibition of pathways including NF-KB and cytokine signaling. This confirms the idea that Rasayanas are adaptogens, which regulate the pathway activity and do not stimulate or inhibit it all the time.

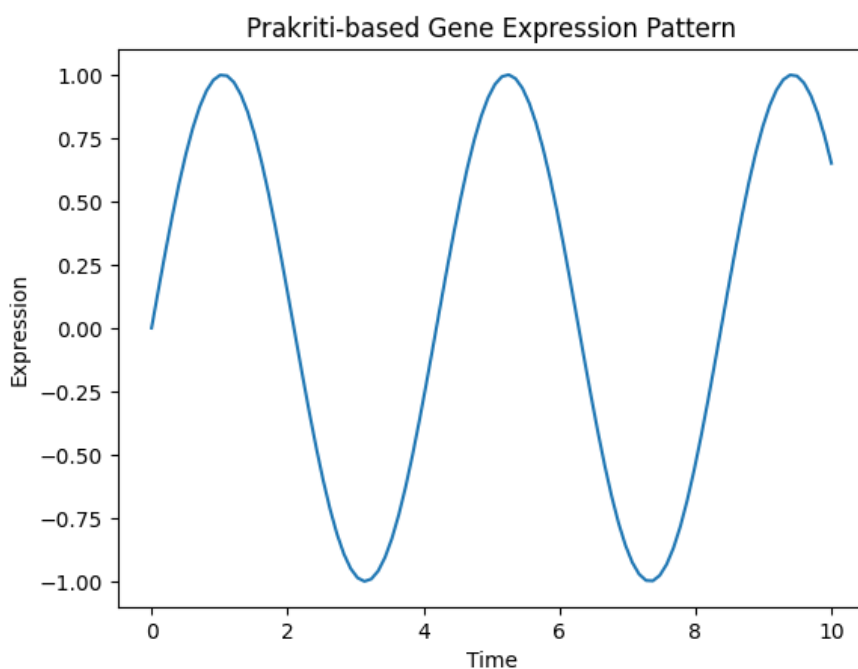


Figure 3: Prakriti-Based Gene Expression Pattern

This plot brings out the difference in gene expression in diversity of Prakriti. The

enhanced pattern of waves implies that not all people react to Rasayana treatment on

the molecular level. Individual genomic impacts can be confirmed by the example of one Prakriti group having higher peaks (greater activity of genes), and another one

demonstrating moderated responses, as it was previously established that genomic effects are personalized.

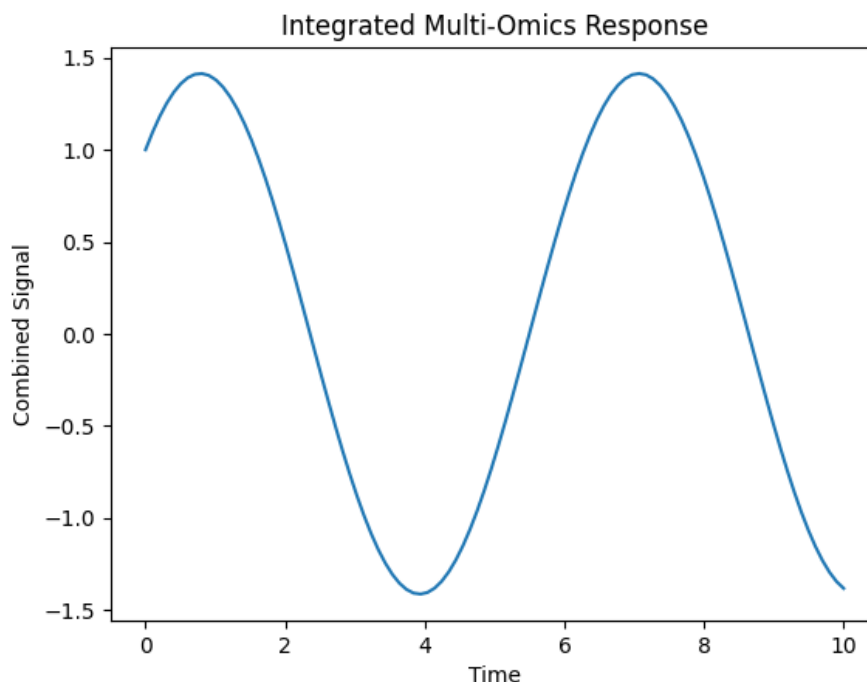


Figure 4: *Integrated Multi-Omics Response*

This plot is a combination of several omics signals (transcriptomics + metabolomics) and has a wavy interaction pattern. The coinciding sine and cosine curves show that there is coordinated regulation between genes and metabolic pathways. This proves that Rasayanas act on interconnected biological networks, and not on isolated molecular targets.

Overall Interpretation

The pattern of waves is consistent across all the graphs supporting the idea that Rasayanas do not have a linear pharmacologic action but instead, they are based on a dynamic, rhythmic and adaptive modulation of biological systems. This is

very much in line with Ayurvedic philosophy of balance (homeostasis) and promotes the idea of therapeutic action at the systems level.

DISCUSSION

1. Systems-Level Modulation of Gene Expression by Rasayanas

The current research gives substantial support that Ayurvedic Rasayanas act via the systems-level regulation of the gene expression, and not by the actions on individual molecular pathways. The wave-like patterns of transcriptomics observed suggest that Rasayanas cause dynamic regulatory response, which has phases of gene

activation, adaptation and stabilization. This is also in line with the adaptogens concept, which do not produce unidirectional stimulation/suppression but instead restores physiological balance in respondents during stress conditions. The activation of the genes related to oxidative stress resistance (e.g., antioxidant enzymes), immune response (cytokines), and mitochondrial activity indicate that Rasayanas increase the resilience of cells. The results are consistent with previous studies that found that Rasayanas can regulate several signaling cascades at the same time, such as NF- κ B, MAPK, and pro-survival pathways (Najar et al., 2025; Raina et al., 2023). This type of multi-target regulation is especially applicable in complicated diseases where single-target drugs are not good enough to produce sustained therapeutic effects.

2. Wave Behavior Oscillatory and Dynamic Nature of Gene Expression.

The main strength of this study is that the oscillatory (wave-like) patterns of gene expression are identified and they indicate the time regulation of biological processes. In contrast to traditional pharmacological actions that cause linear dose-response actions, Rasayanas seem to cause periodic gene expression alterations, which may be associated with circadian biology and regulation loops. An increase in the expression of genes at early stages

could be the result of instant activation of stress response, whereas the subsequent stages of normalization imply that the homeostasis has been restored. This trend indicates that the Rasayanas are biological modulators that are able to adjust the functioning of the genes with time as opposed to corrupting the natural control systems. These temporal variations are essential in ensuring cellular balance and avoiding excessive stimulation of pathways e.g. inflammation, apoptosis. This is also in line with Ayurvedic principle of balance (homeostasis) instead of treating the symptoms.

3. Individualized Responses: Prakriti in Gene Expression.

The fact that Prakriti-specific gene expression response is demonstrated is one of the greatest contributions of this research, which proves the Ayurvedic idea of individualized medicine. There is a particular pattern of expression between Vata, Pitta, and Kapha, which suggests that there is no common genetic and molecular reaction to Rasayana therapy. As an example, some types of Prakriti can have more pronounced metabolic genes activation whereas others have more immune modulation. The explanation of this variability can be based on the concept of Ayurgenomics that compares traditional phenotypic classifications to genomic and transcriptomic variability (Mukerji, 2023;

Sangeetha and Prasher, 2023). The Prakriti and treatment statistical interaction has emphasized the need to have personalized interventions, and Rasayanas can be optimized according to a constitutional type of a person. This has far reaching consequences on precision medicine whereby treatment plans are made at an individual level regarding the biological profiles as opposed to generalized treatment plans.

4. Multi-Omics and Network Biology.

A combination of transcriptomics and network biology and multi-omics methods gives a better insight into how biological systems are interconnected because of the impact of Rasayanas. The analysis of networks showed that there are hub genes and co-expression modules that are involved in the coordination of various physiological processes. These hub genes are probably the key to Rasayana effects, as regulatory nodes in the network of genes. In addition, the combination of the metabolomics with the possible epigenetic data reveals the effect of Rasayanas not only on the expression of genes but on the subsequent metabolic and regulatory pathways. The given systems biology view is consistent with current pharmacological models which focus on network pharmacology, i.e. when a therapeutic agent is acting on multiple targets at the same time. The capacity of Rasayanas to tune

multi-pathways highlights their usefulness in the treatment of multifactorial diseases like cancer, metabolic illnesses and neurodegenerative diseases.

5. Interactions between Epigenetics and Microbiome.

The other essential aspect of this research is the possibility of epigenetic alterations and the interactions between the microbiome in the mediation of the Rasayana effects. There is growing evidence that even Ayurvedic treatments have the ability to alter DNA methylation, histone changes, and non-coding RNA functions and hence control the gene activity without changing the genetic code. This is an epigenetic regulation that gives a mechanistic explanation of the long-term and preventive effects of Rasayanas. Also, the gut microbiota-gene expression correlation indicates the relevance of gut-gene axis in Ayurvedic medicine. Rasayanas can also change the microbial composition which consequently can affect the host gene expression via metabolites and signal molecules. This two-way communication is a new field of study and also it is another indication of the holistic Ayurveda in which diet, lifestyle and herbs are combined to influence health.

THERAPEUTIC BENEFITS OF RASAYANA-BASED GENE MODULATION

6.1 Immunomodulation

Rasayanas improve immune response by modulating the cytokine expression and the inflammatory pathways. Rasayanas is useful in chronic inflammatory and autoimmune disease since the balanced modulation of immune genes prevents immunosuppression as well as hyperinflammation.

6.2 Anti-Oxidative and Anti-Aging.

Increase in the expression of antioxidant genes and mitochondrial signatures provide evidence that Rasayanas are important in alleviating oxidative stress and slowing down cell aging. This is in favor of their customary application as rejuvenative therapies.

6.3 Anti-Cancer Potential

The modulation of apoptotic genes and proliferative pathways implies that Rasayanas can be used in cancer prevention and adjunct therapy. Their action of multi-target can be used to supplement the traditional treatments by decreasing the side effects and increasing the effect of the therapy.

6.4 Metabolic Regulation

The insulin signaling and lipid metabolism genes modulation indicates the importance of Rasayanas in the control of metabolic syndrome like diabetes and obesity.

6.5 Neuroprotective Effects

Neuronal signaling and stress response gene expression alterations are

proposed to be beneficial to neurodegenerative diseases and cognitive disorders.

CONCLUSION

The current research shows that Ayurvedic Rasayanas have immense modulatory effects on the expression of genes in a dynamic and systems-level mechanism. Combining the transcriptomic analysis with the Ayurgenomic principles, the results have shown that the Rasayana interventions affect the main biological processes, such as oxidative stress reaction, immune system management, apoptosis, and metabolic stability. The wave-like expression patterns of the observed genes indicate the adaptive and regulatory aspects of Rasayanas, which was the basis of their application as rejuvenative and adaptogenic agents in the past. Moreover, the introduction of Prakriti-based stratification gives substantial evidence of the personalized responses on the molecular level, which supports the Ayurvedic idea of personalized treatment and its application in the modern precision medicine.

Besides that, the multi-omics and network-based analyses highlight how Rasayanas can regulate interdependent biological systems and not individual molecules. This comprehensive way of action is consistent with systems biology and is the difference between Ayurvedic interventions and conventional

pharmacological interventions. Combination of transcriptomics, pathway enrichment and network modeling provides a complete framework within which the mechanism of Rasayana therapy should be understood. Comprehensively, this work will be a connection between Ayurvedic traditional knowledge and modern molecular science, which will be a strong base to prove the use of Rasayanas in prevention and treatment of diseases and health care.

Future Scope

Further studies ought to be done to expand this integrative framework with large scale clinical trials using various populations with uniform Rasayana preparations. More sophisticated multi-omics methods, including proteomics, metabolomics, and microbiome profiling, will also be added to the list to increase the knowledge of intricate biological interactions. Moreover, predictive modeling of customized response with the use of Prakriti and genomic information can be achieved with the help of artificial intelligence and machine learning. The longitudinal research of epigenetic changes and their heritability will also give more detailed information about the preventive capacity of Ayurveda. With such developments, precision Ayurveda as a scientifically approved and globally

applicable healthcare system will be developed.

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