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MOLECULAR MECHANISMS OF RASAYANA THERAPY FOR HEALTHY AGEING: A SYSTEMATIC REVIEW OF GENOMIC AND EPIGENOMIC EVIDENCE

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ABSTRACT

Healthy ageing has become a global health priority owing to the increasing burden of age-related disorders and the need for interventions that preserve physiological and cognitive function. Rasayana therapy, a rejuvenative branch of Ayurveda, has gained scientific interest for its potential to modulate molecular mechanisms associated with ageing. To systematically synthesize the available genomic, transcriptomic, epigenomic, proteomic, metabolomic, and microRNA evidence describing the molecular mechanisms through which Rasayana therapy promotes healthy ageing. A systematic literature review was conducted following the PRISMA 2020 guidelines. Electronic databases (PubMed, Google Scholar, and ScienceDirect) and manual reference screening were searched for English-language studies published between 2016 and 2026. Original research investigating Rasayana interventions and reporting molecular evidence related to healthy ageing was included. Methodological quality was assessed using the Joanna Briggs Institute (JBI) Critical Appraisal Tools, and findings were synthesized using a qualitative narrative approach. Fourteen studies met the eligibility criteria. The evidence demonstrated that Rasayana therapy modulates multiple interconnected molecular pathways involved in healthy ageing, including FOXO, SIRT1, AMPK, Akt–mTOR, and NRF2. The interventions consistently enhanced antioxidant defence, mitochondrial bioenergetics, genomic stability, neuroprotection, synaptic plasticity, and immune regulation. Advanced molecular technologies, including transcriptomics, epigenomics, proteomics, metabolomics, and microRNA profiling, provided comprehensive mechanistic insights and highlighted the multi-target nature of Rasayana therapy. Rasayana therapy promotes healthy ageing through coordinated regulation of multiple longevity-associated molecular pathways. Although current evidence is predominantly preclinical, the consistent molecular findings support further clinical validation and the integration of multi-omics approaches to facilitate translational research and precision medicine applications.

KEYWORDS: Rasayana therapy; Healthy ageing; Multi-omics; Molecular mechanisms; Ayurveda.

1. INTRODUCTION

Ageing of the population has turned out to be one of the most important demographic transitions the world has ever encountered as the prevalence of chronic illnesses, neurodegenerative diseases, metabolic malfunction and deteriorating physiological strength have increased. Healthy ageing is not only focused on the lifespan, but also the functional ability, cognitive, and quality of life; it involves maintaining cellular and molecular homeostasis during ageing process. On the biological level, ageing is defined by interrelated processes, such as oxidative stress, mitochondrial dysfunction, genomic instability, epigenetic changes, chronic inflammation, and cellular repair malfunctions. A number of highly conserved signalling networks, including FOXO, SIRT1, AMPK, mTOR, and NRF2, coordinate these processes and control the metabolism, stress resistance, antioxidant defence, and longevity of cells. In turn, the fact that these molecular pathways can be modulated by interventions, which have become the focus of scientific interest, has led to the development of potential strategies to enhance healthy ageing and prevent age-related diseases (Saifi et al., 2022).

In the Ayurveda, Rasayana therapy is a medical practice in traditional medicine aimed at increasing longevity, mental acuity, immune response, tissue repair as well as physiological strength. Despite centuries of Rasayana formulations, recent biomedical studies have started to pay more attention to understanding their molecular mechanisms through the application of more sophisticated experimental methods. Recent studies of medicinal plants that are widely used in Rasayana preparations, such as Tinospora cordifolia and Centella asiatica have shown that they can regulate antioxidant

defence systems, mitochondrial activity and regulate gene expression related to cellular protection and metabolic homeostasis (Kundu et al., 2024). Equally, genome sequencing and functional genomic studies of medicinal plants have been useful in shedding light on the biosynthetic pathways involved in the production of bioactive phytochemicals which also lead to their therapeutic effects (Mahajan et al., 2024).

The fast progress in high throughput molecular technologies has also changed the research of traditional medicinal interventions. RNA sequencing-based genome-wide transcriptome profiling has made it possible to identify differentially expressed genes in stress adaptation, metabolism and cellular signalling in a comprehensive way, which has led to a better understanding of complex biological responses to medicinal plants (Chandrakanth et al., 2024). Similarly, de novo transcriptomic studies of Rasayana-associated medicinal plants have shown that there are many genes related to secondary metabolite biosynthesis and regulatory processes related to pharmacological actions (Kapoor et al., 2023). In more recent times, combined transcriptomic and metabolomic studies have facilitated the concomitant assessment of gene expression and the production of metabolites, which have systems-level evidence of the molecular pathways by which medicinal plants act on their biological actions (Wan et al., 2024).

Although there is an increasing amount of molecular research, the existing evidence is still disjointed with regard to different experimental models, medicinal plants and specific biological pathways. Moreover, the technological progress of nanotechnology and other more advanced approaches to translational biomedicine is ever-expanding the potential of natural products, but these technological achievements have not been systematically applied to Rasayana research to provide a multi-layered molecular structure of healthy ageing (Husain et al., 2023). Anti-ageing effects of classical Rasayana interventions have also been shown to have promising effects in model organisms, such as better lifespan and physiological resilience, but these have been typically reported on a case-by-case basis without being incorporated into a wider mechanistic picture (Pathak et al., 2016). In the same manner, research that has investigated Tinospora cordifolia and Withania somnifera have found evidence of cognitive, neuroprotective, and age-related neurological dysfunction improvements by the modulation of oxidative stress and neuronal signalling pathways, however, these studies remain scattered across individual studies, not synthetically created (Bhandari et al., 2022; Kumar et al., 2021). As a result, a comprehensive systematic review that combines genomic, transcriptomic, epigenomic, proteomic, metabolomic and microRNA data to elucidate the molecular pathways by which Rasayana therapy facilitates healthy ageing is lacking.

More recent experimental data has been showing that Rasayana medicinal plants act upon their effects in a concerted manner by modulating a set of biomolecular pathways as opposed to a single biological target. Indicatively, Bacopa monnieri has been demonstrated to stimulate neuronal survival, mitigate glutamate-induced toxicity, and extend life in experimental models, indicating its possible use in promoting healthy ageing by combining molecular pathways (Brimson et al., 2020). Similarly, conserved signalling pathway activation, including AMPK, has been linked to decreased neuronal death, enhanced mitochondrial fitness, and cognitive impairment survival, highlighting the value of pathway-based studies in ageing studies (Li et al., 2024). Thus, a systematic synthesis of the existing multi-omics evidence is needed to bridge the traditional Ayurvedic knowledge to modern molecular biology, discovery of common biological pathways underlying Rasayana therapy, and a scientific basis of future translational research, biomarker discovery, and application of Rasayana interventions to healthy ageing studies.

This systematic review aimed at synthesising in detail the existing genomic, transcriptomic, epigenomic, proteomic, metabolomic and microRNA data on the molecular processes by which Rasayana therapy helps to maintain a healthy ageing process. Further, the purpose of the review was to summarize the key signalling pathways modulated by Rasayana interventions, assess the effects of Rasayana interventions on the oxidative stress, mitochondrial activity, DNA repair, neuroprotection, immune regulation, and synaptic plasticity, summarize the molecular technologies used to study the mechanisms, and evaluate quality and risk of bias of the studies included.

2. METHODOLOGY

2.1 Study Design

This paper is a systematic literature review (SLR) that adheres to the guidelines of Preferred Reporting Items of Systematic Reviews and Meta-Analyses (PRISMA 2020). The purpose of the review was to identify, evaluate, and synthesize the published evidence on the molecular mechanisms of Rasayana therapy in healthy ageing, focusing on the gene expression, transcriptomics, epigenetics, microRNA regulation, proteomics, mitochondrial activities, and ageing-related molecular pathways, such as FOXO, SIRT1, mTOR, AMPK, and NRF2.

2.2 Literature Search Strategy

The search in literature was performed with the help of Google Scholar, PubMed, and ScienceDirect and supplemented with the manual search of reference lists of the relevant articles. It was searched only in English-language publications, published between the year 2016 and 2026. The Boolean operators were used to combine key words in Rasayana therapy, healthy ageing, neuroprotection, longevity, and molecular mechanisms (AND and OR). A representative search strategy was: ("Rasayana") AND (neuroprotection OR longevity) AND ("gene expression") AND (microRNA OR transcriptomics OR epigenetics). Minor modifications to the search syntax were made where necessary to suit individual database requirements.

2.3 Eligibility Criteria

Original research papers published from January 2016 through December 2026 on the topic of Rasayana therapy and/or Rasayana medicinal plants reporting molecular findings on healthy aging, longevity, neuroprotection, oxidative stress, mitochondria, gene expression, transcriptomics, epigenetics, proteomics, microRNAs, and aging-related signaling pathways (FOXO, SIRT1, mTOR, AMPK, and NRF2) were considered for inclusion in the review. Studies utilising in

vitro, animal or human experimentation design approaches were included if the results generated from the experiments contributed to the goals of the review with respect to the molecular aspects. Reviews, systematic reviews, meta-analysis, conference abstracts, editorial, book chapter, letter to editor and non-English papers were excluded from consideration.

2.4 Study Selection

All the records retrieved were imported into a reference database and duplicates were eliminated and then screened. Titles and abstracts were filtered based on the predetermined eligibility criteria and the potentially relevant articles were filtered based on their full-text. Titles, abstracts, and full-text articles were independently screened by two reviewers, and any disagreements were resolved through discussion to reach consensus. The selection of the studies was carried out based on the PRISMA 2020 recommendations, and the entire workflow is illustrated in the PRISMA flow diagram.

2.5 Data Extraction

The extracted data included the author and year of publication, Rasayana intervention, experimental model, molecular techniques applied, and the main findings related to oxidative stress, mitochondrial function, neuroprotection, epigenetic regulation, and other molecular mechanisms associated with healthy ageing. Data extraction was performed independently by two reviewers using a standardized extraction format, and discrepancies were resolved through consensus.

2.6 Risk of Bias Assessment

The quality of methodology and risk of bias of the included studies were evaluated with the Joanna Briggs Institute (JBI) Critical Appraisal Tools, and the most appropriate checklist was chosen depending on the type of study design of each article included. The appraisal appraised important methodological areas, such as the clarity of the research purpose, the suitability of the study design, sample selection, intervention or exposure measurement, outcome measurement, statistical analysis, bias or confounding control, and the consistency of findings with the reported research. The quality evaluation (in general) was included in the synthesized evidence interpretation. In order to have the appraisal results presented in a visual manner, the risk-of-bias assessment was summarized as a traffic-light plot with Risk-of-bias Visualization (robvis). The overall certainty of evidence was qualitatively considered based on the consistency of findings, methodological quality, and reproducibility across the included studies.

2.7 Data Synthesis

Thus, a qualitative narrative synthesis was done to compare the included studies based on the Rasayana intervention under investigation, molecular mechanisms, ageing-related signalling pathways, and genomic, transcriptomic, epigenetic, proteomic and microRNA results that were relevant to healthy ageing with special reference to the FOXO, SIRT1, mTOR, AMPK and NRF2 pathways.

3. RESULTS

3.1 Study Selection

The systematic literature search revealed 440 records, including 423 records discovered by searching electronic databases and 17 records discovered by screening references manually. Having eliminated 63 duplicate records, 377 studies were left to undergo screening of titles and abstracts. Then 313 records were removed since they failed to meet the predetermined eligibility criteria. The other 64 full-text articles were evaluated on eligibility with 50 articles excluded due to reasons such as not being relevant to the Rasayana therapy, or lack of molecular results, or inappropriate study design or lack of methodological details. Lastly, 14 studies met all the inclusion criteria and were included in the qualitative synthesis. The PRISMA flow diagram (Figure 1) shows the detailed study selection process.

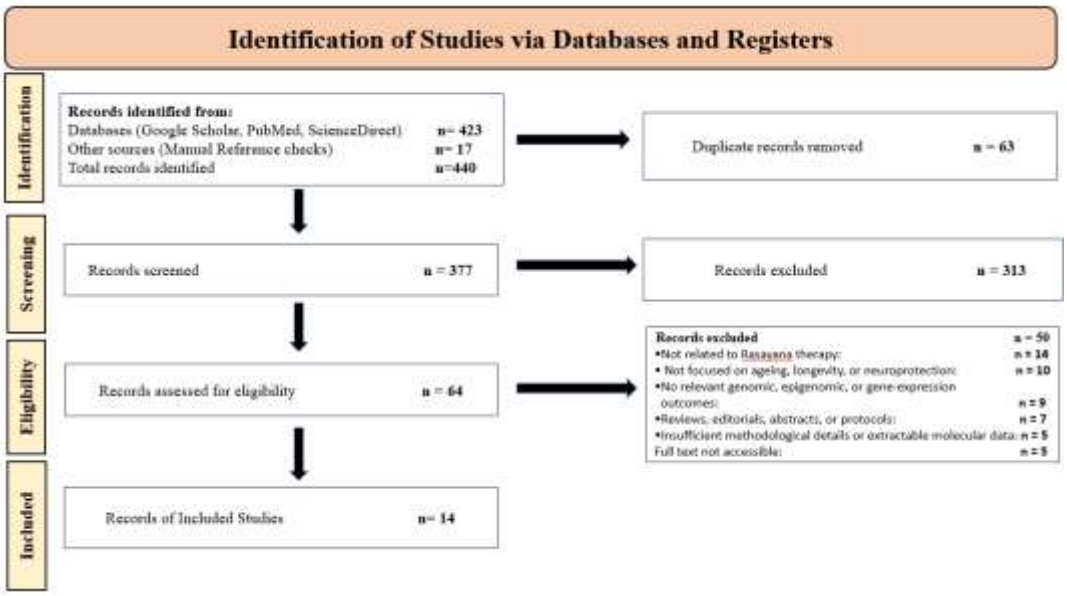

Figure 1: PRISMA flowchart

3.2 Characteristics of Included Studies

The 14 studies incorporated, published between 2016 and 2026, explored the molecular mechanism of Rasayana therapy in various experimental models, such as Drosophila melanogaster, rodent models, neuronal cell lines, ex vivo tissues, and human subjects. The trials considered Rasayana formulations including Amalaki Rasayana, Bacopa monnieri, Centella asiatica, Withania somnifera, Clitoria ternatea and Medhya Rasayana. A large variety of molecular methods, such as RT-qPCR, western blotting, DNA methylation analysis, RNA sequencing, microRNA sequencing, LC-MS/MS proteomics, metabolomics, Seahorse mitochondrial assays, and electrophysiological validation were used to examine ageing-related mechanisms. Generally, the evidence showed consistent evidence that Rasayana therapies regulate oxidative stress, mitochondrial activity, DNA repair, neuroprotection, synaptic plasticity, immune regulation, and key longevity-related signalling pathways, such as FOXO, SIRT1, AMPK, Akt-mTOR, and NRF2. Table 1 provides a summary of the detailed characteristics of the included studies.

Table 1. Characteristics of the Included Studies

Author (Year)	Rasayana Intervention	Experimental Model	Molecular Technique(s)	Main Findings
Dwivedi & Lakhotia (2016)	Amalaki Rasayana	*Drosophila melanogaster*	RT-qPCR; oxidative stress assays	Improved stress tolerance and antioxidant defence.
Preethi et al. (2016)	*Bacopa monnieri*	Experimental memory model	DNA methylation analysis; molecular assays	Regulated epigenetic mechanisms associated with memory enhancement.
Kumar et al. (2017)	Amalaki Rasayana	Rat cardiac hypertrophy model	RT-qPCR; western blotting; biochemical assays	Enhanced mitochondrial function and cardiac metabolism.
Leung et al. (2017)	*Bacopa monnieri*	Human SH-SY5Y neuroblastoma cells	RNA sequencing; pathway analysis	Altered neuronal gene expression and stress-response pathways.
Gray et al. (2017)	*Centella asiatica*	Hippocampal neuronal cell model	Mitochondrial respiration and oxidative stress assays	Improved mitochondrial bioenergetics and reduced oxidative stress.
Matthews et al. (2019)	*Centella asiatica*	5XFAD mouse model	RT-qPCR; western blotting; immunohistochemistry	Activated NRF2-mediated antioxidant signalling.
Zweig et al. (2021)	*Centella asiatica*	5XFAD mouse model	Molecular assays; pharmacological inhibition	Enhanced NRF2-dependent antioxidant defence and synaptic function.
Bhat et al. (2023)	Ayurveda intervention	Human participants	Genome-wide DNA methylation profiling	Identified differential DNA methylation associated with immune regulation.
Sharma et al. (2025)	*Withania somnifera*	Human SK-N-SH neuroblastoma cells	Whole-transcriptome RNA sequencing	Modulated neurodegeneration-related gene expression.
Fanibunda et al. (2025)	*Withania somnifera*	Rat cortical neurons and neocortex	RT-qPCR; western blotting; ELISA; Seahorse respirometry	Enhanced mitochondrial biogenesis and neuronal energetics.
Boondam et al. (2025)	Standardized *Centella asiatica* (ECa 233)	SH-SY5Y cells and rat hippocampal slices	LC-MS/MS proteomics; western blotting; electrophysiology	Improved mitochondrial metabolism and synaptic plasticity.
Prasad et al. (2025)	*Clitoria ternatea*	Neuronal cell model	DNA damage assay; western blotting; immunocytochemistry	Enhanced DNA repair and neuronal differentiation.

Maruthiyodan et al. (2026)	Medhya Rasayana	Alzheimer's disease mouse model	RNA sequencing; LC-MS metabolomics	Reduced neuroinflammation through multi-omics regulation.
Mohamed Puhari et al. (2026)	*Withania somnifera*	Human SK-N-SH neuroblastoma cells	microRNA sequencing; pathway analysis	Identified microRNA-mediated regulation of longevity-related pathways.

3.3 Genomic, Epigenomic, and Multi-Omics Evidence

The studies included showed that Rasayana therapy has an effect on healthy ageing via various layers of molecular control, such as genomic, epigenomic, transcriptomic, proteomic, metabolomic and post-transcriptional actions. Epigenetic studies found changes in DNA methylation in neuronal functionality and immune response, and transcriptomic studies found extensive changes in oxidative stress, cellular metabolism, inflammation, and neurodegeneration-related genes. This evidence was further extended by recent studies to include microRNA sequencing, quantitative proteomics, and integrated multi-omics methods which provide detailed insights into the intricate molecular networks underlying Rasayana-mediated healthy ageing. In general, the results suggest that the effects of Rasayana interventions are mediated by various interrelated biological mechanisms and not a single molecular target. Table 2 summarizes the major molecular processes that were found in the studies included.

Table 2. Major Molecular Mechanisms Identified Across the Included Studies

Biological Process	Principal Molecular Targets	Functional Significance	Representative Studies
Epigenetic regulation	DNA methylation, transcription factors	Regulation of neuronal function and immune responses	Preethi et al. (2016); Bhat et al. (2023)
Transcriptomic regulation	Differentially expressed genes, cytokine signalling	Modulation of metabolism, oxidative stress, and neurodegeneration	Leung et al. (2017); Sharma et al. (2025); Maruthiyodan et al. (2026)
Post-transcriptional regulation	microRNAs, FOXO, PI3K/AKT, MAPK, TP53	Regulation of longevity, apoptosis, and neuronal survival	Mohamed Puhari et al. (2026)
Proteomic remodelling	Mitochondrial and synaptic proteins	Enhanced bioenergetics and synaptic function	Boondam et al. (2025)
Antioxidant defence	NRF2, HO-1, NQO1, GCLC, SOD	Protection against oxidative damage	Dwivedi & Lakhotia (2016); Matthews et al. (2019); Zweig et al. (2021)
Mitochondrial bioenergetics	SIRT1, PGC-1α, NRF1, TFAM, ATP	Improved mitochondrial function and energy metabolism	Gray et al. (2017); Fanibunda et al. (2025); Boondam et al. (2025)
DNA repair	Base-excision repair, γH2AX	Maintenance of genomic stability	Prasad et al. (2025)
Synaptic plasticity	BDNF, CAMK2A, SNAP25, LTP	Enhanced learning, memory, and neuronal connectivity	Preethi et al. (2016); Fanibunda et al. (2025); Boondam et al. (2025)

3.4 Ageing-Related Signalling Pathways

A number of the conserved signalling pathways during healthy ageing were invariably altered throughout the participating studies. The most common pathways reported were FOXO, SIRT1, AMPK, AktmTOR and NRF2, which are all important in the regulation of oxidative stress, mitochondrial homeostasis, cellular metabolism, protein synthesis, and cell survival. Despite variations in the Rasayana interventions acting on different molecular targets, the overall data indicates the convergence on shared longevity-related networks of signalling that preserves cellular homeostasis and enhances resilience to age-related stress. These interrelated pathways offer a mechanistic model to the effects of Rasayana therapy on anti-ageing. Table 3 presents the key signalling pathways indicated in the covered studies.

Table 3. Major Ageing-Related Signalling Pathways Modulated by Rasayana Therapy

Signalling Pathway	Representative Molecular Targets	Primary Biological Role	Representative Studies
FOXO	FOXO-associated miRNAs	Longevity, apoptosis, oxidative stress resistance	Mohamed Puhari et al. (2026)
SIRT1	SIRT1, BDNF, PGC-1α, NRF1, TFAM	Mitochondrial biogenesis and energy metabolism	Fanibunda et al. (2025)

AMPK	phospho-AMPK, PRKAA1	Cellular energy sensing and metabolic regulation	Kumar et al. (2017); Boondam et al. (2025)
Akt–mTOR	Akt, mTOR	Protein synthesis, mitochondrial metabolism, neuronal plasticity	Boondam et al. (2025)
NRF2	NRF2, HO-1, NQO1, GCLC	Antioxidant defence and redox homeostasis	Matthews et al. (2019); Zweig et al. (2021)

3.5 Oxidative Stress and Mitochondrial Function

Oxidative stress and mitochondrial functioning regulation was the most frequently reported biological process in all the included studies regardless of the Rasayana intervention or experimental model used. All of this evidence indicated that Rasayana therapies reduced intracellular oxidative stress by lowering reactive oxygen species and lipid peroxidation and increasing endogenous antioxidant defence mechanisms. The enhanced expression of antioxidant mediators such as superoxide dismutase (SOD), Hsp27, HO-1, NQO1, and GCLC were indicators of the activation of the intrinsic cellular defence systems that prevent oxidative damage and maintain redox homeostasis (Dwivedi & Lakhotia, 2016; Matthews et al., 2019; Zweig et al., 2021).

In addition to antioxidant protection, several studies uniformly revealed an improvement in mitochondrial bioenergetics after Rasayana treatment. Experimental data showed that there was an increase in ATP production, basal and maximal mitochondrial respiration, oxidative phosphorylation, an increase in the quantity of mitochondrial DNA, mitochondrial biogenesis was stimulated by regulating the signalling pathways of SIRT1, PGC-1, NRF1 and TFAM. Increased mitochondrial homeostasis was seen to be a shared feature of *Centella asiatica* and *Withania somnifera* treatments despite the differences in their antioxidant activities, suggesting that the maintenance of mitochondrial homeostasis is a widespread molecular pathway of mitochondrial ageing (Rasayana therapy) (Gray et al., 2017; Fanibunda et al., 2025; Boondam et al., 2025).

3.6 Neuroprotection, DNA Repair, and Synaptic Plasticity

Another robust result that crossed the entire studies included was neuroprotection, whereby Rasayana therapies affected various events that contributed to neuronal survival, genomic stability, and cognitive abilities. Transcriptomic and proteomic studies continuously revealed the regulation of genes and proteins related to the neurodegenerative pathways, neuronal differentiation, and cell survival. Moreover, Clitoria ternatea stimulated the repair of the base-excision DNA damage, mitigated the DNA damage caused by γH2AX, and altered proteins related to the neuronal differentiation and autophagy, which demonstrated the enhanced preservation of genomic integrity in the conditions of the cellular stress (Leung et al., 2017; Sharma et al., 2025; Prasad et al., 2025; Boondam et al., 2025).

The fact of better neuronal functioning was further supported by the research on the synaptic plasticity and interaction between mitochondria and neurons. Withania somnifera increased neuronal resilience by BDNF-SIRT1 signalling, promoting neuronal mitochondrial functionality and neuronal stress resistance, and proteomic analyses of Centella asiatica revealed important modulation of synaptic proteins, such as CAMK2A and SNAP25. The additional electrophysiological validation supported the increased hippocampal long-term potentiation, which is the functional evidence that molecular modifications were manifested in the form of ameliorated synaptic plasticity. Collectively, these results propose that Rasayana therapy can promote healthy ageing of the brain by orchestrating the maintenance of genomic stability, neuronal survival, mitochondrial activity, and neuronal communication (Fanibunda et al., 2025; Boondam et al., 2025).

3.7 Emerging Multi-Omics Technologies

The articles included revealed a gradual transformation of the traditional molecular studies to the multi-omics methods of cognizing how the Rasayana therapy works. This development allowed thorough characterization of ageing related molecular changes at several levels of biological systems, giving more understanding of the intricate state of regulatory systems that lead to healthy ageing. Together, these methodological improvements have solidified the mechanistic evidence of Rasayana interventions and have provided a better ground to do future translational and precision medicine studies. The table of contents of molecular technologies utilized in the studies included is presented in Table 4.

Table 4. Molecular Technologies Used to Investigate the Mechanisms of Rasayana Therapy

Methodological Category	Techniques Applied	Primary Molecular Information Generated	Representative Studies
Gene-expression analysis	RT-qPCR	Quantification of ageing- and stress-related genes	Dwivedi & Lakhotia (2016); Kumar et al. (2017); Fanibunda et al. (2025)
Protein-expression analysis	Western blotting, ELISA, Immunofluorescence	Validation of signalling proteins and molecular targets	Kumar et al. (2017); Fanibunda et al. (2025); Boondam et al. (2025)
Epigenomic profiling	DNA methylation analysis	Identification of ageing-associated epigenetic modifications	Preethi et al. (2016); Bhat et al. (2023)
Transcriptomic profiling	RNA sequencing	Genome-wide differential gene-expression analysis	Leung et al. (2017); Sharma et al. (2025)

Post-transcriptional profiling	Next-generation microRNA sequencing	Identification of miRNA regulatory networks	Mohamed Puhari et al. (2026)
Proteomic profiling	LC–MS/MS proteomics	Global protein-expression profiling and pathway analysis	Boondam et al. (2025)
Integrated multi-omics	Transcriptomics–metabolomics	Correlation of gene expression with metabolic pathways	Maruthiyodan et al. (2026)
Functional mitochondrial assays	Seahorse extracellular flux analysis, ATP assays	Assessment of mitochondrial respiration and bioenergetics	Fanibunda et al. (2025); Boondam et al. (2025)
Functional validation	Electrophysiology, pharmacological inhibition	Validation of neuroprotective and signalling mechanisms	Zweig et al. (2021); Fanibunda et al. (2025); Boondam et al. (2025)

3.8 Risk-of-Bias Assessment

Based on the study design, methodological quality of the included studies was evaluated based on the relevant Joanna Briggs Institute (JBI) critical appraisal tools. On the whole, the risk of bias was mostly low, with most studies fulfilling most of the quality assessment criteria. Few studies were found to have moderate risk of bias owing to minor methodological limitations, mostly associated with the reporting of sample selection and methodological information. However, the general quality of methodology in the studies included justifies the general quality of the synthesized evidence. The risk-of-bias evaluation of the included studies in detail is presented in Figure 2.

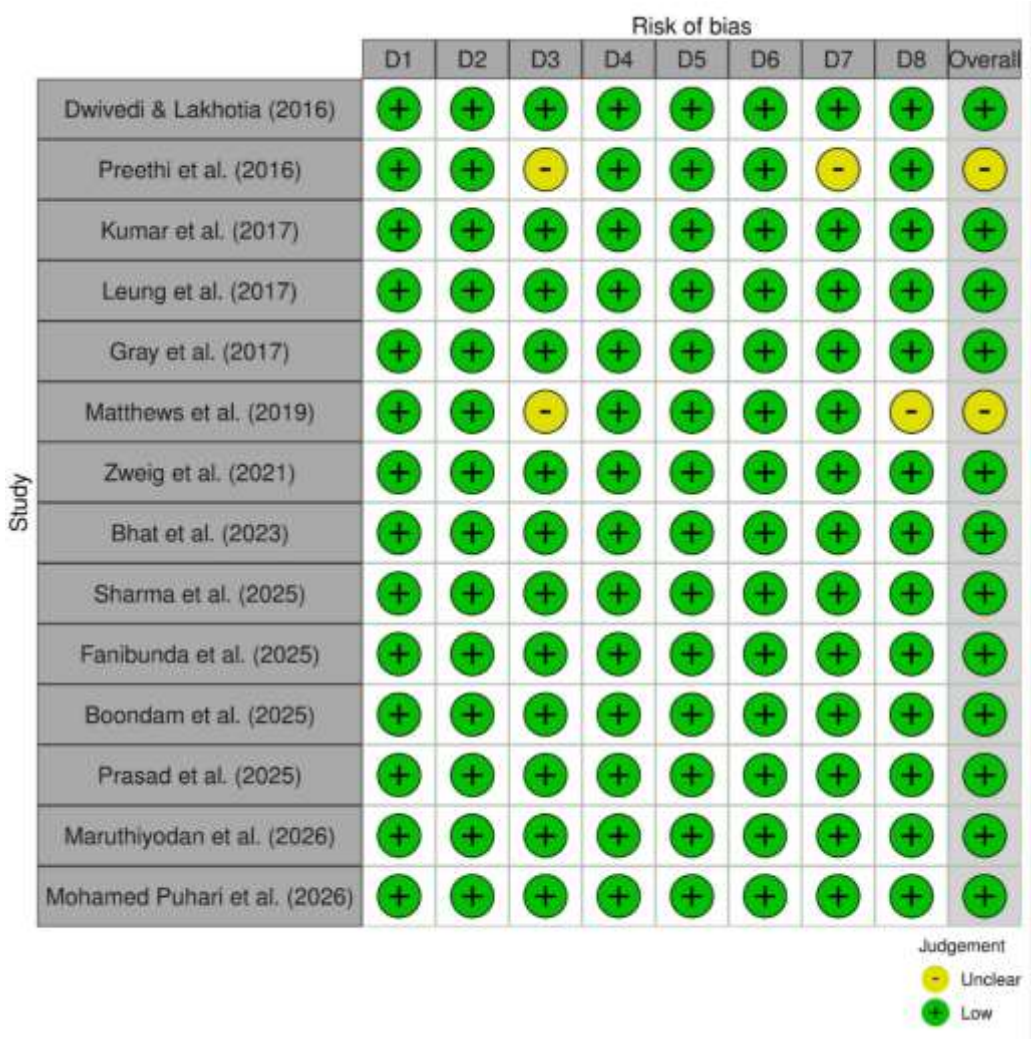

Figure 2. Risk of bias assessment (D1 = Clearly defined research objective; D2 = Appropriate study design and experimental model; D3 = Adequate sample selection/allocation; D4 = Clearly described intervention/exposure; D5 = Valid and reliable outcome measurement; D6 = Appropriate statistical analysis; D7 = Control of bias/confounding; D8 = Conclusions supported by the reported results)

4. DISCUSSION

The current systematic review was a synthesis of existing genomic, transcriptomic, epigenomic, proteomic, metabolomic, and microRNA data on the molecular mechanisms underlying Rasayana therapy that leads to healthy ageing. Together the

results suggest that Rasayana interventions have their biological effects via the coordinated action of several interconnected signalling pathways and not at one molecular target. The studies included all showed that the regulation of oxidative stress, mitochondrial bioenergetics, DNA repair, neuroprotection, synaptic plasticity, and immune homeostasis is mediated by FOXO, SIRT1, AMPK, Akt-mTOR, and NRF2 pathways. The growing use of transcriptomics, epigenomics, proteomics, metabolomics and microRNA profiling also exemplifies the shift in classical pharmacological studies towards systems-level molecular characterization, thus offering a more powerful mechanistic rationale of the traditional notion of Rasayana as a rejuvenative treatment.

Based on the synthesized evidence, the molecular effects of Rasayana interventions appear to be mediated through the coordinated regulation of interconnected signalling pathways rather than a single molecular target. Activation of FOXO, SIRT1, AMPK, Akt–mTOR, and NRF2 pathways collectively contributes to enhanced antioxidant defence, improved mitochondrial function, maintenance of genomic stability, neuroprotection, and immune homeostasis, thereby supporting healthy ageing. This integrated mechanistic framework highlights the multi-target nature of Rasayana therapy and provides a biological basis for its rejuvenative effects.

The present review findings align with the past literature that has shown that Rasayana medicinal plants have geroprotective potential. Vittal and Vinciguerra (2025) pointed out that Withania somnifera facilitates healthy ageing by regulating oxidative stress, mitochondrial activities, neuroprotection, and chronic inflammation, which is closely related to the molecular processes reported in the current synthesis. Likewise, Gu et al. (2022) highlighted the key contribution of PI3K/AKT signalling pathway to mediate the neuroprotective effects of conventional herbal medicines, which results in the regulation of Akt -mTOR-related signalling by a multitude of Rasayana interventions. Iantomasi et al. (2025) also identify the significance of epigenetic control and non-coding RNAs in age-related diseases, and their results on oxidative stress and the role of microRNA in molecular regulation are consistent with the combined genomic and post-transcriptional data found in this review. Moreover, NF-KEB-mediated mechanisms of withaferin A to regulate cellular senescence were also shown by bioinformatic and experimental studies by Bhargava et al. (2019), which supports the idea that Rasayana compounds regulate several longevity-related signalling cascades. Previous experimental research also supported the current review by demonstrating that withaferin A controls apoptosis, cellular stress responses, and genomic integrity via various molecular mechanisms (Yu et al., 2017; Chang et al., 2017).

The results have significant implications on the field of biomedical research and integrative medicine. Translationalally, overlapping of several molecular pathways is an indication that Rasayana treatment can be a multi-target intervention, which can independently and concomitantly regulate oxidative stress, mitochondrial metabolism, genomic stability and neuronal activity. The integration of multi-omics technologies has enhanced the biological insights of Ayurvedic traditional formulations as well, through a measurable molecular data that can attest to their claimed rejuvenative action. This evidence can help in biomarker discovery, precision medicine strategies and scientifically integrating Rasayana therapy into research of healthy ageing and preventive healthcare strategies.

In spite of these strengths, there are a number of limitations that can be identified. The studies included were very heterogenous concerning Rasayana interventions, experimental models, molecular techniques, and outcome measures, making it impossible to make direct comparisons across studies. Majority of the evidence was based on in vitro research and animal experiments; however, well-designed studies that involve advanced molecular studies in human clinical studies are limited. Variations in the methodological procedures and reporting guidelines further diminished the possibility of quantitative syntheses and meta-analysis. This means that the conclusions must be put into perspective based on the preclinical evidence and exploratory clinical evidence that exists.

The future studies should focus on large-scale, expertly designed clinical studies involving transcriptomics, epigenomics, proteomics, metabolomics, and microRNA profiling aimed at confirming the molecular mechanisms revealed in experimental studies. Improved comparability across studies will require standardized Rasayana formulations, harmonized molecular outcome measures and longitudinal study designs. Moreover, the combination of the multi-omics analysis using artificial intelligence, systems biology, and precision medicine strategies can help gain a better understanding of the multifaceted molecular pathways of a healthy ageing process that is promoted by the Rasayana and can be implemented to develop evidence-based individual interventions.

5. CONCLUSION

This study was a synthesis of the current genomic, transcriptomic, epigenomic, proteomic, metabolomic, and microRNA studies to understand what the molecular pathways of Rasayana therapy in healthy ageing are. Taken together, the results suggest that Rasayana interventions do not have a single therapy target, but instead a concerted action on a variety of interconnected molecular pathways to have their biological effects. In all the included studies, Rasayana therapy had a consistent effect on key longevity-related signalling pathways, as well as FOXO, SIRT1, AMPK, Akt -mTOR and NRF2, leading to improved antioxidant defence, mitochondrial bioenergetics, genomic stability, neuroprotection, immune regulation, and synaptic plasticity. The review also points out the growing use of sophisticated molecular methodology, such as transcriptomics, epigenomics, proteomics, metabolomics, and microRNA profiling that have significantly enhanced the mechanistic basis of conventional Ayurvedic rejuvenative treatments. The general concordance in molecular evidence in the three types of Rasayana interventions despite the fact that most of the evidence is mostly based on the experimental and preclinical studies gives a solid biological explanation as to why they may have a role to play in healthy ageing. However, more rigorous methodological standardization and a well-designed clinical study with integrated multi-omics techniques is needed to substantiate these molecular mechanisms in humans. In general, the review not only connects traditional Ayurvedic knowledge with modern molecular biology but also offers an overall synthesis of the existing evidence and forms a scientific basis of future translational research, the discovery of biomarkers, and the integration of Rasayana therapy into the strategies of healthy ageing and precision medicine.

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