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Review

Ayurveda and *in silico* Approach: A Challenging Proficient Confluence for Better Development of Effective Traditional Medicine Spotlighting Network Pharmacology

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ABSTRACT Coalescence of traditional medicine Ayurveda and *in silico* technology is a rigor for supplementary development of future-ready effective traditional medicine. Ayurveda is a popular traditional medicine in South Asia, emanating worldwide for the treatment of metabolic disorders and chronic illness. Techniques of *in silico* biology are not much explored for the investigation of a variety of bioactive phytochemicals of Ayurvedic herbs. Drug repurposing, reverse pharmacology, and polypharmacology in Ayurveda are areas *in silico* explorations that are needed to understand the rich repertoire of herbs, minerals, herbo-minerals, and assorted Ayurvedic formulations. This review emphasizes exploring the concept of Ayurveda with *in silico* approaches and the need for Ayurinformatics studies. It also provides an overview of *in silico* studies done on phytoconstituents of some important Ayurvedic plants, the utility of *in silico* studies in Ayurvedic phytoconstituents/formulations, limitations/challenges, and prospects of *in silico* studies in Ayurveda. This article discusses the convergence of *in silico* work, especially in the least explored field of Ayurveda. The focused coalesce of these two domains could present a predictive combinatorial platform to enhance translational research magnitude. In nutshell, it could provide new insight into an Ayurvedic drug discovery involving an *in silico* approach that could not only alleviate the process of traditional medicine research but also enhance its effectiveness in addressing health care.

KEYWORDS Ayurveda, *in silico* approach, confluence, challenge, effective therapeutics

Ayurveda is renowned and one of the oldest traditional systems of medicine in the world. Traditional medicines have good capabilities to treat many chronic diseases such as arthritis, asthma, cancer, diabetes, etc. Traditional Chinese medicine (TCM) and Indian Ayurvedic medicine (IAM) are increasing their acceptance in the United States.[1] Unfortunately, this precious gift from our ancestors is sprawling due to a lack of scientific proof and validation of various concepts of Ayurveda. Therefore, it is imperative to further explore traditional medications like Ayurveda to get insights into their active constituent, activity, characterization, elucidation of the mode of action, etc. These arduous issues can unravel if research in the field of Ayurveda begins on the interface of *in silico* biology. *In silico* denotes work performed on the computer about biological experiments. It is a paradigm shift from *in vitro* and *in vivo* drug discovery process to *in silico* drug discovery screening, from "wet lab" to "dry lab", human resources to computational resources, manpower to automation, animal or cell models to computer models, and from highly expensive to economical research. Computer-based high throughput screening methods truly suit the idea of refinement, reduction, and complement the mechanism-based research in Ayurveda.[2]

In silico biology is burgeoning globally, covering the development of software to capture, analyze, and integrate biological/medical data acquired from multiple sources. The use of unexplored information over Ayurvedic medicine using computational models could create a

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hypothesis, their validation, and ultimately advancement in Ayurvedic therapeutics.[2] *In silico* methods includes software, databases, and various tools (quantitative structure-activity, relationship pharmacophores, homology model and other molecular modeling approaches, machine learning, data mining, network analysis, etc.) that could retrieve various meaningful information quickly in an unexplored area of Ayurveda.[3] *In silico* methods are primarily used in tandem with *in vitro* databases to create and test the model, such model has been successively used in the discovery and optimization of the novel molecule having affinity to the targets. It is a trans-disciplinary branch that embodies multiple domains of science and technology targets from screening and identifying the bio-actives and target genes responsible for the cure and cause of disease respectively.[4] Current drug discovery research usually takes 10–12 years and costs 1.5 billion USD. If we start with the screening of 1 million compounds, only 250 compounds enter the pre-clinical phase followed by 10 of them entering the clinical trial and only one qualifies for clinical uses[3] and it consumes huge time and money. Computational screening of compounds provides support for quick identification and lead development. It can reduce the time of lead discovery and cut short the pipeline of drug discovery[5,6] having the potential to get informed with many issues (predicting drug toxicity, activity, interaction, mechanism, maximizing efficacy, etc.).[7]

There are many reviews available on the core Ayurvedic medicine domain to date. But the dedicated discussion on Ayurveda insights and their *in silico* confluence is missing. The 21st century is considered the beginning of a new era, receptive to healthcare philosophy and a recent decade has witnessed much landmark development in natural product investigation with the tools of *in silico* biology for quick deliverables. Through this review, we highlighted the necessity to link-up the Ayurveda with *in silico* biology, the international scenario of Ayurveda practices and the need for Ayurinformatics, and the conjugation of *in silico* tools in the domain of Ayurveda for immense exploration of Ayurveda core concepts. This review article also discusses the challenges and future prospects of *in silico* studies in Ayurveda that is also not reviewed precisely and properly earlier.

Linking Up Ayurveda Concept with *in silico* Biology

Ayurveda is one of the world's most ancient methodological life knowledge system which is used for holistic human well-being natural remedies, more preventive rather than curative, etc. Over time, natural products and traditional medicine have grabbed the attention of the pharmaceutical industry. Among 74 antihypertensive drugs, 48 are derived from the natural product, and 7 out of 10 anti-migraine drugs originated from traditional medicine.[8] Anti-malarial drug artemisinin and its analog can be considered a boon from traditional medicine to modern medicine. Looking into primordial clues and tremendous scope, the drug discovery process could be accelerated by the traditional uses of Ayurvedic medicine. To speed up this process Ayurveda must be explored extensively *in silico* domain because it would be done in a time-efficient manner for the identification of cost-effective novel and selective leads from natural resources. *In silico* approach provides a platform for drug target identification, virtual screening, structure activity relationship, and toxicity prediction, from the available information of Ayurveda. We can effectively evaluate the *in silico* relevance of traditional uses of Ayurvedic medicine.

In silico approach is a quick method to screen a large number of phytoconstituents of Ayurvedic plants to understand the initial mode of action that could be further proved by pre-clinical studies. *In silico* biology is a very vast area that is classified in Appendix 1. Bioinformatics domain of *in silico* biology is further subdivided into many branches (Ayurinformatics, Clinical informatics, Epidemiology informatics, Immunoinformatics, Pharmacoinformatics, Public health informatics, etc.) and tools (molecular docking, molecular dynamic simulation, ADMET, etc.). Bioinformatics study not only gives the idea of ligand-receptor interaction but also provides information about nature of ligand (hydrophobic, hydrophilic, amphipathic), target/receptor proteins (drug target), drug-target interaction (antagonist or agonist), target-ligand interface, binding sight of receptor and mechanism, mode of biding, properties of binding, specificity of ligands (mono or multi), other various bondings and interactions i.e. drug-drug interactions, drug-metabolic interactions, multi-mode ligand-receptor interactions, hydrogen bonding, electrostatic interactions, intermolecular interactions, hydrophobic interactions, various specificities like ligand-specific binding, ligand binding affinity, ligand activation, ligand conformers, ligand specificity, ligand-protein conjugates, ligand-directed signaling, ligand-selective activity, various molecular level information viz. molecular conservation,

molecular recognition, lipophilicity, lipophobicity, molecular scaffolds, polar/non-polar contacts, multivalent ligand-receptor interactions, multiple ligand recognition, nuclear receptors, quantum interaction, universal ligand, single and multiple targeting additionally. Extracting this information from *in silico* approach is very useful to enhance understanding. This area of *in silico* biology is known as computational drug design that includes computational modeling, chemocentric informatics, computer-assisted design, evolutionary design of ligands, drug target identification, virtual screening, molecular docking, ligand-induced structural polymorphism, pharmacophore modeling and mapping, quantitative structure-activity relationships (QSAR), rational approach to drug design, structure-based design and simulation.

In summary, a bioinformatics resource for *in silico* analysis could give more thoughts on the diversity/potential of Ayurveda and gaining momentum to explore more *in silico* work under the branch of Ayurinformatuics but unfortunately, very little *in silico* work has been done on the interface of Ayurveda concepts. Although a high surge *in silico* drug discovery work was seen during the coronavirus disease 2019 (COVID-19) pandemic. The researcher screened a large number of phytochemicals to obtain potent moiety against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to the effective role of traditional medicine in treatment.[9] A lot of works are warranted that further emphasize the integration of *in silico* and Ayurveda and the need for Ayurinformatics studies.

International Scenario of Ayurveda Practices and Need of Ayurinformatics

Ayurveda has a fair capability to treat diseases (chronic/non-communicable and communicable). Unfortunately, due to the lack of scientific validation of Ayurveda concepts, this precious gift of knowledge for human health given as heritage from our ancestors is trailing.[10] Globally a few research groups are working on the Ayurvedic and modern medicine interface. Focus on Ayurvedic education and research is lacking (even in India where Ayurveda originated).[11-13] A brief overview of the status of Ayurveda research globally is discussed continent-wise: (1) Asia: Ayurveda has a good base in Asian countries. A large number of Asian people rely on Ayurvedic treatment. Some of the Asian countries where Ayurveda is gradually attracting the scholars and populations are China, Japan, South Korea, Sri Lanka, Thailand, etc. Ayurveda has gradually attracted the attention of scholars and yoga practitioners in China. (2) Africa: The Nelson Mandela School of Medicine, South Africa offers Ayurveda courses in affiliation with the Ayurveda faculty, Natal University, South Africa. Ayurveda practice is recognized by various Boards of the South African Ayurveda Liaison Committee and the African Allied Health Professional Act. (3) Australia: An International Congress of Traditional Asian Medicine (ICTAM) was held in Canberra in 1979 and followed by the establishment of the Australian School of Ayurveda in Adelaide with the help of experts from Indian Universities. Later in 1994, the Australasian Academy of Natural Medicine was established. But the recognition and legal status of Ayurveda as a medical system is yet far from reality. (4) America: The National Institute of Ayurvedic Medicine (NIAM) was established in 1982 in the USA. It is recognized as the largest and most authentic resource of information on Ayurveda in the United States. Some Latin American countries like Costa Rica, Guatemala Nicaragua, and Venezuela, have legally approved the use of Ayurveda medicines. (5) Europe: European Academy of Ayurveda has been established in 1993 and contributed to the development of Ayurveda both by professionalizing and educational schemes. European Union (EU) directive for traditional herbal medicines was brought into force in 2005. Each member of EU passed its own regulation for the trade of traditional medicines within this directive. A large number of EU tourists to India seek treatment with Ayurveda for a wide range of diseases and also purchase Ayurvedic medicines.

Central government agencies of India like Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy (AYUSH; www.ayush.gov.in), Central Council for Research in Ayurvedic Science (CCRAS), and the Indian Council for Medical Research (ICMR) emphasize Ayurveda practice for the traditional approach. Centre for Complementary and Integrative Health (CCIH) Pune, India is working on a combinatorial interface network pharmacological evaluation of the immunomodulatory activity of Ayurvedic medicine along with other investigations for natural phosphodiesterase inhibitors, neurodegenerative disorders, natural product-based drug repurposing and so on.[14] Uttaranchal Ayurvedic Medical College, Dehradun, India had done documentation of medicinal plants in Lacchiwala Range, Dehradun Forest Division of Uttarakhand Province because it is a huge natural reservoir.[15] IMPPAT, a curated database of Indian Medicinal Plants,

Phytochemistry, and Therapeutics, was developed by the Institute of Mathematical Science (IMSc), Homi Bhabha National Institute (HBNI) Chennai, India. It has a manually curated database of 1,742 Indian Medicinal Plants, 9,596 phytochemicals, and 1,124 therapeutic uses spanning 27,074 plant-phytochemical associations and 11,514 plant therapeutic associations.[16] The National Medical Plant Board (NMPB) of India has established the Indian Medical Plants Database, containing 7,263 species of Indian medicinal plants (http://www.medicinalplants.in/). There are 2,559 kinds of Ayurveda, 2,267 kinds of Sida, 1,049 kinds of Unani, 460 kinds of homeopathy, and 6,403 kinds of folk medicine.

Ayurinformatics research studies can play a significant role in the development of new Ayurvedic drug moiety before the preclinical and clinical studies because drug development with a known approach is an intense, lengthy, and interdisciplinary venture. Therefore, need for Ayurinformatics is emphasized. Various Ayurinformatics tools and techniques could co-operate a vital role to confirm the drug target and drug discovery before going to *in vitro/in vivo* studies. Recent progress in Ayurinformatics approaches might help the drug design and development process.[5-7,17-19] What is now more important, the researcher might judge this domain regarding effectiveness, time-cost, easiness, and many other properties of Ayurinformatics.

The Ayurvedic treatment uses a set of complex pharmaceutical combinations. So, to know the proper therapeutic value/clinical efficacy of the poly-herbal/Ayurvedic formulations needs modern tools of bioinformatics (Appendix 1) before going into *in vitro/in vivo* studies because quick screening and identification of effector molecules can be done fast by implementing *in silico* approach in poly-herbal/Ayurvedic formulations. Herb-drug interaction caused by enzymatic inhibition and induction can be quickly identified by *in silico* screening. So research professionals should start research in the field of *in silico* biology and Ayurveda to quickly get more leads. Herbs-herb, herb-concept, and concept-concepts-based models of Ayurveda are warranted to develop better lead for treatment (Appendix 2). No doubt, the speed is slow but research has been started at the interface of Ayurveda and *in silico* approach. In the next section, we have described some important *in silico* studies done on Ayurvedic plants/medicines that further emphasize various interesting findings.

In silico Studies on Ayurvedic Medicines: A Promising Confluence

The pharmacological action and mechanism of traditional medicine are not limited to improving immunity and increasing adaptability. The mechanism of Ayurveda is not limited to improving immunity but also has many pharmacological effects, such as liver protection, anti-cerebral ischemia, anti-virus, and so on. Phytoconstituents fight against common infections through receptors toll-like receptors (TLRs), pattern recognition receptors (PRRs), Fc receptors, B/T cell receptors, killer activated, and killer inhibitor receptors (KARs and KIRs), and complement receptors. Phytoconstituents are supposed to have the ability to protect the body (by mediating cytokine/antibody production) against external pathogenies that induce disease.

Recently, a trend towards the use of *in silico* biology (molecular modeling, docking, simulation, etc.) to understand the Ayurveda concept has gained significant momentum which revolutionized the drug discovery and development process. Though *in silico* work is not an answer to all questions, it may save time, short-circuit the process of drug development, and shed some light on the mode of action of phytoconstituents to escalate Ayurveda practice with a better understanding (Appendix 3). The literature discussed here is concerned with a targeted subject that reflects a good orientation in eclectic consequences. It is very difficult to summarize the whole work of an area, therefore, we have discussed some important and recent *in silico* work that has been done previously with the phytoconstituents of Ayurvedic plants (Appendix 4).[20-38]

Profiles based on the receptor binding affinity, D-lysergic acid amide (LSA) and lysergic acid diethylamide (LSD) of *Argyreian nervosa* (Burm.f.) Bojer have been compared by Paulke, et al.[20] *In silico* study has shown that the aglycones and their derivatives of Ayurvedic plant *Bacopa monnieri* L. Pennell have a good binding affinity, good central nervous system (CNS) drug-like properties, and had good blood-brain barrier penetration.[21] Molecular docking followed by molecular simulation revealed the anti-inflammatory property of methyl gallate [extracted from a plant *Bergenia ligulata* (Wall.)] by inhibiting the enzyme lipoxygenase.[22] The anti-inflammatory property of α-mangostin (αMN, a xanthone present in *Garcinia mangostana* Linn. and widely used as functional food supplement in Ayurveda) was reported

by Mohan, et al[23] in 2018 using *in silico*, *in vitro*, and *in vivo* approaches. Recently strong interactions of some potential compounds from an Ayurvedic plant *Capparis zeylanica* L. leaf extract was identified with antimicrobial properties by a computational approach. These compounds were (3E)-N-(3,4 dichlorophenyl)-3-(propionylhydrazono) butanamide, heptadecanoic-margaric acid, and 5-(3-fluorophenyl)-7-nitro-1,3-dihydro-2H-1,4-benzodiazepine-2-one and molecular docking has shown more specificity toward the microbial receptors and the highest fitness score.[24] *Cassia auriculata* L. phytoconstituents reconcile glucose/lipid metabolism via the PI3K signaling pathway, and influence AKT, thereby causing insulin secretion/insulin sensitivity in peripheral tissues and modulating immunometabolic pathways.[25] Phytocompounds [(Z)-9,17-octadecadienal, kaempferol-3-monoglucoside] of *Clitoria ternatea* L. show the inhibitory effect with enzyme monoamine oxidase and acquire an *in silico* approach in rejuvenating Ayurvedic neurodegenerative medicine.[26]

Oil of *Cocos nucifera* L. has high medicinal use in the Ayurvedic system. *In silico* analysis has been done to study the effect of coconut oil phytoconstituents [medium chain fatty acids (MCFAs) and phenolic compounds].[27] MCFAs and phenolic compounds of coconut oil target the molecules of the polyol pathway. This study revealed that lauric acid interacts with aldose reductase (AR) and dipeptidyl peptidase-4 (DPP-Ⅳ) of the polyol pathway and inhibits the activity of AR. A molecular docking study revealed that guggulsterone-E binds to the minor groove of DNA and interacts through a single hydrogen bond formation between the hydroxyl group of guggulsterone-E of adenosine (A6) and nitrogen (N3).[28] Roots of *Inula racemosa* Hook. f. (used in Ayurveda in India) was shown as a cardioprotective via targeting estrogen receptors alpha (ERα) and beta (ERβ).[29] The anticancerous activity of piplartine (an alkaloid of *Piper longum* L.) was analyzed by molecular docking and some *in vitro* studies and potent interactions were shown with the corresponding target receptors.[30] Secondary metabolites of *Piper longum* (piperine and its derivatives such as piperonylic acid, piperic acid, and piperonal) inhibited lipoxygenase enzyme by binding at its active site.[31] *In silico* analysis of 3'-O-neopentyl moiety of salacinol showed a potent α-glucosidase inhibitor from Ayurvedic traditional medicine 'Salacia' (*Salacia reticulata* Wight).[32] The molecular dynamic simulations performed by Ahmad, et al[33] predicted the possible binding mode of pinitol (bioactive compound of *Saraca asoca* (Rox.) Willd.) with tumor necrosis factor alpha (TNF-α) and showed anti-inflammatory activities against carrageenan-induced edema. This study also accentuates the probable reason behind the synergistic effect of (+)-pinitol along with β-lactam antibiotics. Resins of *Semecarpus anacardium* L. were identified as 2 selective inhibitors of acetylcholinesterase inhibitor by Adhami, et al.[34] The findings of molecular docking done by Sudeep, et al[35,36] showed that withanolides of Viwithan have a good binding affinity with the antiapoptotic protein myeloid leukemia-1 (MCL-1) against liver hepatocellular carcinoma. Chandran and Patwardhan[37] have reported the immune modulation mechanism of 5 bioactive compounds of *Withania somnifera* that are capable of regulating 15 immune system pathways through 16 target proteins by protein-protein interactions and bioactive-target. *In silico* analysis of *Withania somnifera* natural compound withaferin-A was done by Grover, et al[38] and showed a rationalized ability of withaferin-A that alters and inhibits the nuclear factor kappa B (NF-κB) signaling pathway.

These *in silico* studies at the interface of Ayurveda phytoconstituents further warrant the need for pre-clinical and clinical research of the active compounds to increase the understanding of the development of effective therapeutics in future in Ayurveda. We have discussed some investigations done in the Ayurveda domain with *in silico* tools and discussed future possibilities of their confluence to prove the theory/concept of Ayurveda. Ayurveda follows the theory of tridosha (3 regulatory functional factors of the body), dhatu (major structural component of the body), panchmahabhoot, nidan panchak, and other unique concepts. Cellular models for *in silico* study are available but Ayurveda needs concept-based models. Models which can satisfactorily fit the varying concepts viz Anupanas (adjuvant), aushadh sevan kala, the dominance of dosha changes, Ritucharya, Dincharya. We need such platforms for effective and authentic rationalization of the Ayurveda concept and *in silico* approach could help well in its initiation research in Ayurveda. Authors attempted to compile some studies of drugs starting from their traditional uses to their validation through *in silico*, *in vitro*, *in vivo*, and clinical studies (Appendix 5).[39-84] The identification of various phytoconstituents and their target-based mode of action performed in these studies further demonstrate the utility of *in silico* study with Ayurvedic phytoconstituents for the discovery of novel and effective

drug leads. These results advocated for the exploration of several multi-plant formulations that could be considered as a potential source for novel treatments in Ayurveda.

Challenges, Limitations, and Future Prospects

During the COVID-19 pandemic, *in silico* study has witnessed a huge surge but there are still some challenges such as a large number of databases providing information regarding potential targets, pathways but with variations, which further need optimization. All the compounds are not suitable for computer-aided drug design because of their special structure properties, the accuracy of various algorithms, and maturity of the model needs to be further improved. Limited scientific evidence has restricted Ayurveda's global use and acceptance. Though research is an essential and compulsory part of Ayurveda practices still it is in the infancy stage and needs acceleration. Information technology in the last decades got a very big boost. With the advancement in science and technology, the progress in the field of drug discovery and development is amplifying every day. Drug discovery is heading from the concept of "single-drug single-target" to "single-drug multiple-targets".[85] Ayurveda strongly believes in this principle in terms of polypharmacology. The root of Ashwagandha (*Withania somnifera*) acts as a nervine tonic, aphrodisiac, diuretic, narcotic, anti-helminthic, astringent, and thermogenic drug.[86] *In silico* studies can contribute to understanding the combinatorial effect of multiple bio-actives in a single herb (Appendix 5). However, it needs the synchronization and systematic integration of data obtained from different disciplines of science.

Traditional medicines like TCM or IAM have multiple therapeutic ingredients. Phytochemicals of various ingredients of a traditional bind with multiple targets associated with multiple diseases. This is also very challenging but the network pharmacology domain of bioinformatics could be helpful to shed a light on it. TCM is known for mechanistic complexity and holistic approach. In the last decade, TCM has synergistically utilized computational biology for the pharmacological understanding of phytoconstituents and their cross-talks within the formulation. The combination of simulated compounds and accurate prediction of the targets helped in the primary scrutiny of TCM formulations. Network pharmacology leads to the demonstration of various activity targets of phytochemicals and complex relationships between the compounds. PubMed and China National Knowledge Infrastructure (CNKI) database reflect a surge *in silico* research in TCM after 2010. TCM research based on network pharmacology and computational biology encompasses broad range of topics such as studying the pharmacological effect of drugs, their mode of action, analyzing the various theories of TCM, and exploring the application of TCM. With the implementation of network pharmacology, syndrome research has boosted, disease-phenotype-gene-drug model is used effectively to understand the ZHENG (syndrome) through multitarget-multicomponent-multidisease approach. Some other prediction methods used in TCM research are machine learning algorithms, pharmacophore modelling, pharmacological structural genomics, virtual screening, drug characterization methods, molecular docking, and molecular dynamic simulations. The conjunction of TCM and computational biology have added knowledge in cancer immunotherapy, cardiovascular diseases, hepato-protection, breast cancer, chronic bronchitis, pulmonary diseases, depression, and type Ⅱ diabetes mellitus.

Network pharmacology study of Triphala [*Emblica officinalis* (Gaertn.), *Terminalia chebula* (Retz.), *Terminalia bellarica* (Gaertn.) Roxb.] showed that it can work against 15 types of disease involving 74 different disease indications through modulation of 31 protein targets against cancer, cardiovascular disease, neurodegenerative disorder, and metabolic disorders.[39] This *in silico* finding (done on the interface of Ayurveda) further indicated to extend research (pre-clinical and human) and evaluate Triphala for such new disease indications and its mode of action. In this way, an *in silico* study could help in the extension of the traditional medicine scope and newer disease coverage. In fact *in silico* studies deserve a well-acclaimed root space needed to extend the effective Ayurveda framework in the future. The situation of Ayurveda is like an iceberg, factors visible on the surface are less as compared to underlying factors which may lead to the formation of the effective Ayurveda framework (Appendix 6). Broadly, talking about external factors first is proactive policy push and research support while the second is institutional strengthening, inclusive acts, and regulation. *In silico* could initiate, generate, and strengthen the evidence for the Ayurvedic system of medicine. Many interesting leads are originated from an *in silico* study done with Ayurvedic drugs (Appendix 5). One such article by Borse, et al[87] on an *in silico* multi-target approach for Ayurveda botanicals in COVID-19 management supports the utility of multiple botanicals to be screened or trialed for the current pandemic. Although *in vitro*, *in vivo* and clinical studies have already

been done on Ashwagandha (*Withania somnifera*) for immunomodulation and antioxidant effects.[70] Some other Ayurvedic herbs are also undergone clinical trials starting from *in silico* study (Appendix 5). Now Ashwagandha is under clinical trial for COVID-19 patients. These few examples suggest that *in silico* technology has boosted the pace to reach clinical trials. Further standardization of drugs is a valid concern that comprises Pharmacovigilance, Good Agriculture Practices (GAP), Good Manufacturing Practices (GMP), Good Clinical Practices (GCP), etc. (WHO, 2007) to substantiate principle-based practices.[88,89] Along with them, the development of multidimensional and compatible human resources will enhance the overall research capacity of Ayurveda (Appendix 6) in association with *in silico* work.

In silico screening can trace the purposefulness of various combinations of medicines available in Ayurveda literature. Balachaturbhdra Churna containing 4 ingredients (*Cyperus rotundus* L., *Piper longum*, *Aconitum heterophyllum*, *Pistachio integerrima* J.L. Stewart *ex* Brandis) is a commonly used medicine for Atisaar (diarrhoea) in pediatric patients.[90] Literature has given many more individual drugs for diarrhoea control but curiosity arises some questions. Why these 4 herbs as formulation? Why not are other drugs having similar action? Do these ingredients have any molecular agonism, target synchrony, or network pharmacological advantage? Whether they have synergistic action or antagonize the adverse actions of one another? These unanswered questions can be authenticated with the start of *in silico* screening and their result can be validated with the support of Wet-Lab (*in vitro* and *in vivo*) work. Taking every Ayurvedic formulation to the Wet-Lab study in search of rationality behind the above query is an expansive and time-consuming process. Therefore, *in silico* approach can put forward a computational scientific basis to explore and support the classical formulations (combinations of drugs). It is very difficult to perform reverse pharmacology clinical trials or drug repurposing clinical trials, especially in the rare disorders and younger age groups (pediatric age) due to ethical issues and hard to obtain consent from parents, so *in silico* drug screening data can be put strong support to research asking for clinical trials in these fields.

All over the globe, metabolic disorders and chronic diseases patients do not rely only on a single therapy. They also consume traditional medicine although not disclosed to their physician, and it causes herb-drug interaction. Quercetin (phytochemicals of an herb) has been identified as an inducer of cytochrome resulting in herb-drug interaction.[91] Conventional pharmacokinetics deals with drug-drug interaction while interactions between the herb and drug have also been reported creating new space and scope for research to study the phytoconstituent-drug interaction. Both modalities (drug and herb) can be effectively used in a conjoint treatment strategy.[92] A plausible combination of herb-drug interaction can lead to the development of more effective and potent new chemical entities to fight against many complex diseases. On this front, *in silico* herb-drug interaction study could be a better and more economical tool for such initial insights and will be the hallmark of tomorrow's biomedicine.

Ayurveda researchers need basic learning to make optimal use of medical informatics for the upwelling of the ancient system of medicine in a productive way. Most Ayurveda scholars, physicians, and researchers are not trained in computers, information technology, bioinformatics, etc., which is a great challenge to explore *in silico* biology in Ayurveda. In this era of science, it is almost impossible to imagine research activity in a lack of information technology. There is a need for serious attempts to introduce lateral postgraduate courses in Ayurveda or research for scholars to equate them with *in silico* understanding. It is also essential to consider *in silico*-based drug discovery/development studies in this sector because it could escalate the trans-disciplinary drug discovery approach in traditional medicine. The lack of professionals having expertise in traditional medicine and information technology is the biggest challenge in this field. Global research collaboration in the field of Ayurveda especially for newer research modalities is well appreciated and required that can address the questions of Ayurveda satisfactorily. The 21st century is an information technology era, hence a better utility and broad application of *in silico* tools are warranted to expand the Ayurinformatics field more for enhancing the understanding of Ayurveda concepts in the modern spotlight.[92]

Toxicity could be exerted by chemical molecules in the living system. Therefore, the toxicological study of a compound is very imperative. Toxicity testing is done on animal models. Animal ethical committee always suggests reducing the number of animals or replacing the animals in safety and toxicity profiling. *In silico* approach is a better alternative for toxicity prediction

based on the chemical structure of a compound.[93,94] Software like TOPKAT (Toxicity Prediction by Komputer Assisted Technology, Accehys Inc Cambridge, UK; a biostatic based and QSAR containing system) is available for conducting toxicological studies. Ayurveda is having a huge collection of formulations in the form of herbal, herbo-mineral, and minerals. *In silico* model can be successfully used to complement relevant safety and toxicity of *in vitro* studies. This interaction can be visualized with the help of toxicity prediction and network-based bioinformatics. Most Ayurveda *in silico* study was done on the inhibitory action of herbal formulations, targeting, etc. (Appendix 4), but a few *in silico* study is available on the herb-minerals preparations of Ayurveda and toxicity issue. Metallic compound interaction impacts the solubility and permeability of the drug. These metal ions could reduce or increase absorption of drugs.[95] Hence *in silico* studies of herbo-mineral preparation is an untouched area that needs to be explored for mineral interactions and toxicity studies in the future (Appendix 2). This can contribute to a better development of core concepts of Rasa shastra i.e. metal based Ayurvedic pharmaceuticals with their mechanistic understanding.

Conclusions

Amid the global crisis of the COVID-19 pandemic, the whole world is looking toward Ayurveda/traditional medicine due to its immunomodulatory and preventive aspects. Appendix 2 has shown the interrelation between these studies and inclusive approaches for convergence of *in silico* approach in Ayurveda. As we know, *in silico* studies can swiftly generate scientific support data to develop lead molecules or substantiate the drug repurposing for prevention, prophylaxis, management of disease, etc. (Appendix 3) and could provide promising leads in public health crises. *In silico* is a quick method to screen a large number of phytoconstituents of Ayurvedic plants to understand the initial mode of action, confirming the lead molecule, and drug target for drug discovery before going to *in vitro*/*in vivo* studies that could be further implemented and utilized in Ayurveda practice. Further *in vivo* and *in vitro* research studies extension have a significant role in the development of a new medicine before the clinical trials. Synergistic computer models, quality database, standardization for testing, and validating the results are some dots of restrictions of *in silico* studies. Hence it is a dire need to strengthen *in vitro* and *in vivo* research infrastructure in the association of *in silico* infrastructure that could be fully able to disclose the complex interplay between drug targets and disease in the future, although some limitation of *in silico* study is associated with availability and applicability, especially in Ayurveda-centric models to complement each other.

Conflict of Interest

Authors declare that they have no conflict of interest.

Author Contributions

RS did knowledge search, manuscript writing, table writing; PKG conceived the manuscript idea, did manuscript writing, manuscript structuring, formatting, editing, and drawing figures; AM performed draft writing and manuscript structuring; AK did manuscript writing, critical analysis, formatting, drawn figures, and correspondence.

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