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REVIEW ARTICLE

Synergistic Potentials of Herbal/Phytomedicines

Arpana Purohit[1], Anoothi Bain[2], Prakhar Nema[1], Sameeksha Jain[1], Harshna Vishwakarma[1], Prateek Jain[1]

[1]Department of Pharmaceeutical Sciences, Adina College of Pharmacy, Sagar, Madhya Pradesh, India, [2]Aizant Drug Research Solutions, Hyderabad, Telangana, India

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ABSTRACT

The utilization of herbal medicines and their combination due to synergistic action especially Chinese traditional and Ayurvedic medicines in treating numerous diseases such as cardiovascular diseases, HIV, and cancer has been longstanding since the primordial times and are still in trend. Synergy research in phytomedicine has been acknowledged as a ground-breaking and crucial activity that is demarcated as the interaction between two or additional agents in order to yield a mutual superior outcome which is larger than the summation of their discrete effects. Various research works unveils the potential of the medicinal herbs with multicomponent possessing synergism. To establish the synergistic potential, its quantification is a major prospect. Furthermore, the investigation of synergy will not only assist the researchers to learn about the new and improved Phyto combinations but also will support in eliminating the probable negative synergistic actions. Further clinical research is needed to verify the reported synergistic mechanisms of various drug combinations. This review discusses the molecular basis, mechanism underlying the effect and challenges of synergistic potentials of herbal/phytomedicines.

Keywords: Computational methodology, Omic technology, phytoconstituents, phytomedicine, protein-protein interaction models, synergy

INTRODUCTION

Synergistic potentials of herbal/phytomedicines have been a vital and an innovative arena of research in the recent years. Synergy can be demarcated as the therapeutic superiority of herbal extract consisting of a blend of bioactive components as compared to the discrete effect of the single component hence, we use the terminology "polyvalent effect" to describe the enhanced effect.[1]

The advanced analytical methods available and the alteration of chemotherapy paradigm from monodrug toward multidrug therapy have escalated the increased research in the field of synergy of phytomedicines. It is significant to determine and verify the exact mechanism of synergistic action. The idea of synergy is broadly grouped into two chief categories - pharmacodynamic and pharmacokinetic synergy based on the approach of activities**.** The former category of synergy explains two or more agents working on a like receptors/biological targets which give rise to improved therapeutic effect due to their positive interactions. The subsequent category is based on the interaction among two or more agents at some stage in their pharmacokinetic process (absorption, distribution, metabolism, and elimination) causing quantitative changes of agents in body and thus the therapeutic efficacy.[2] In the study of underlying mechanism and quantification of the synergistic effect the occurrence of errors is high due to economic factors, choosing optimum analytical method along with ethical issues. Thus, erroneous inferences will be drawn from simple comparative estimation of synergy between combination and discrete components so the

***Corresponding Author:**
Arpana Purohit,
E-mail: tanuprht@gmail.com

development of effective and applied models will be supportive in the analysis and study of synergy of phyto constituents in animals as well as humans.

MECHANISM OF SYNERGY EFFECTS

Based on the current research work ongoing in the field of classical pharmacology, molecular biology and clinical studies the mechanism of synergy has been classified into following types.[3]

1. Synergistic multi-target effects
2. Based on boosted solubility, resorption rate, and enhanced bioavailability – pharmacokinetic or physicochemical effects
3. Interaction of agents with the bacterial resistance mechanisms
4. The neutralization or removal of adverse effects by components present in the extract leading to an enhanced efficacy which can be than accomplished without the use of these agents or manipulations.

Synergistic Multi-Target Effects

When the constituents of a mono-extract impose its action on multiple targets rather than single target so as to produce a synergistic or agonistic action is regarded as the synergistic multi-target effect. However, when the multiple components present in the mono-extract impose its action only on one target only additive or agonistic effect is seen.[4] [Figure 1 and Table 1] represents different phytoconstituents producing multi-target effects.

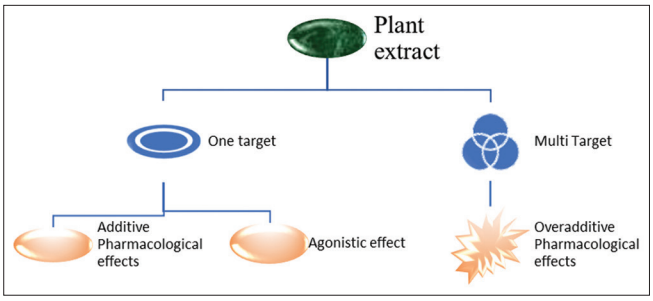

Figure 1: Representation of mono target effect and multitarget effects produced by a mono extract of plant constituting several constituents[2]

Based on Boosted Solubility, Resorption Rate and Enhanced Bioavailability – Pharmacokinetic or Physicochemical Effects

In the arena of phytopharmacology its often observed that certain components of the extract which themselves deficient of any pharmacological action but they enhance the pharmacokinetic properties of the lead compounds such as – solubility, resorption rate, and thus bioavailability ensuing improved performance [Table 2].

Interaction of Agents with the Bacterial Resistance Mechanisms

There are certain agents which when utilized alongside antibiotics are capable of partially or entirely suppressing the resistance mechanism of bacteria. ß-lactam antibiotic penicillin + clavulanic acid: Antagonizes penicillinase resistance.[51] They may achieve this action through following mechanism [Tables 3-5].

The Neutralization or Removal of Adverse Effects by Components Present in the Extract Leading to an Enhanced Efficacy which can be than Accomplished Without the Use of these Agents or Manipulation

When the therapeutic efficacy of the drug is enhanced by using a component present in the plant extract or additionally added that either neutralizes or eliminate the effect of the toxic component as compared to the original drug [Table 6].

METHODS FOR QUANTIFICATION OF SYNERGY

The therapeutic superiority attained due to synergy than the effect of the discrete components makes it an attractive choice in the treatment of numerous diseases. However, to formulate the optimum combination of Phyto leads it's crucial to quantify synergy which definitely is a challenging task. With the initiation of network-based drug discovery, powerful techniques such as system biology and computational technology have arisen in the arena

Table 1: Examples of various herbal phytoconstituents producing multi-target synergistic effects

Serial number	Herbal drug or its phytoconstituents responsible for synergistic action	Synergistic effects	References
1	Cannabis Tetrahydrocannabinol Cannabidiol	Antispastic effects, hallucinogenic, antiemetic, anxiolytic, appetite stimulating, anti-inflammatory and analgesic effects Cannabidiol promotes an increase in the transport of anandamide through the brain membrane	[5-8]
2	Rhizoma coptidis Consist of alkaloids, including palmatine, berberine, epiberberine, coptisine Ferulic acid Compunds of high polarity	Anti-hyperglycemic Anti-inflammatory Anti-Alzheimer Antibacterial Antioxidant Antidepressant	[9]
3	Hypericum (St. John's Wort) Contains hyperforin, hypercines, amentoflavone, rutin, hyperosid, xanthone, proanthocynidines, xanthones, mono and biflavinoids	Antidepressant	[10-15]
4	Iberogast® Composed of extract of nine plants (*Mentha*, *Chamomile*, *Glycyrrhiza*, *Melissa*, *Chelidonium*, *Silybum*, *Calum*, *Angelica*, *Iberis*)	Treats functional dyspepsia and motility related intestinal disorders. Multi-target effect shown by balancing the function of gastrointestinal motility by alleviation of gastrointestinal hypersensitivity by inhibiting inflammation suppressing gastric juice secretion and affects the function of autonomic afferent gastro-intestinal part	[16]

Table 2: Examples of various herbal phytoconstituents producing synergistic effects by boosting pharmacokinetic or physicochemical effects

Serial number	Herbal drug or its phytoconstituents responsible for synergistic action	Synergistic effects	References
1	*A. belladonna* leaf extract Load component - l-hyoscyamin Concomitant compound - flavanol triglycosides	Resorption catalysed	[17]
2	*A. visnaga* Lead component - khellin	Enhanced bioavailability	[18]
3	*H. perforatum* Lead component - hypericin Concomitant compound-polyphenols such as epicatechin, procyanidin, hyperosid, rutin	Enhanced bioavailability	[19]

A. belladonna: Atropa belladonna, *A. visnaga: Ammi visnaga*, *H. perforatum: Hypericum perforatum*

Table 3: Interaction at the active site having a target for intervention

Phytoconstituent and antibiotic combination	Action	References
EGCg and β-lactam antibiotics	Peptidoglycan bacterial cell wall attacked directly or indirectly	[9,20]

EGCg: Epigallocatechin gallate

Table 4: Inhibition of enzymes generated for antibiotic deactivation like -ester or -lactam cleaving enzymes

Phytoconstituent and antibiotic combination	Action against	References
EGCg penicillin	*S. aureus*	[21]

EGCg: Epigallocatechin gallate, *S. aureus: Staphylococcus aureus*

of pharmaceutical industry. Thus, this not only contributes in predicting the novel synergistic combinations but also helps in identifying the molecular basis of the synergistic effect. This may further aid in setting the experimental basis for the study of synergy of phytomedicines [Table 7].

Protein-Protein Interaction (PPI) Model

The theoretical concept is suggestive of the fact that the synergistic drugs have a tendency to affect the target network topological features. The biological molecular model PPI is grounded on the deliberate physical or functional relations between the proteins. This structures the foundation of the interatomic system of the living beings and forms the base of extremely specific molecular networks such as disease specific. Therefore, an experimental score may be determined that is based on the connection of topology along with the centrality of drug targets which aids in the estimation of the probable synergy mechanism [Table 8].

Table 5: Interactions of agents with bacterial resistance mechanisms producing synergistic effect

Serial number	Herbal drug or its phytoconstituents responsible for synergistic action	Synergistic effects	References
1	Load component - berberin and flavonolignan 50 MHC (*H. wightiana*) Concomitant compound - reserpine (*R. officinalis*)	Augments the antimicrobial effect of berberine by inhibition of the efflux of berberine from *S. aureus*	[22-25]
2	*T. vulgaris* Essential oil Antibiotics Synergistic agent - thymol, carvacrol Leaves Action of 5,6,7- trihydroxy flavon baicalein on tetracyclines and β-lactum antibiotics	Enhanced penetration of antibiotics in gram negative bacteria Baicalein affects the peptidoglycan structure of the bacterial membrane and also inhibits the efflux of tetracycline from bacteria	[26,27]
3	*H. lupulus* contains hop xanthohumol, lupulon which acts on antibiotics	Positive synergy effect	[28]

MHC: Methoxy-hydnocarpin, *H. wightiana: Hydnocarpus wightiana, R. officinalis: Rosemarinus officinalis, T. vulgaris: Thyums vulgaris, S. aureus: Staphylococcus aureus, H. lupulus: Humulus lupulus*

Table 6: Examples of neutralization or removal of adverse effects by components present in the extract

Serial number	Herbal drug or its phytoconstituents responsible for synergistic action	Synergistic effects	References
1	*A. belladonna* leaf extract Load component - glycyrrhizin alone Concomitant compound - liquorice root extract	The toxic effects of glycyrrhizin are neutralized like hypertension and edema when extract used as compared to glycyrrhizin alone	[29]
2	*A. radix* and *R. radix*	When used in appropriate dosage increases wound healing in diabetic patients	[30]

A. belladonna: Atropa belladonna, *A. radix: Astragali radix*, *R. radix: Rehmanniae radix*

Table 7: Methods of quantification of synergy

Serial number	Model/method	Description	Additional information	References
1	Combination index	A scientific term to quantitatively depict synergism (CI <1), additive effect (CI=1), and antagonism (CI >1)	Advantages: Its experimentally one of the most practical and demonstrative methods and has no limit for the number of tested combination ingredients Disadvantages: Cannot determine dose response of individual constituents and combination	[31,32]
2	Isobole method	A graphical procedure depending on the position of the dose of combination to the "iso-effect" linear line can either represent additive, synergistic, or antagonistic interactions	Advantages: Experimentally practical and one of the oldest and well-established method Disadvantages: Cannot determine dose response of individual constituents and combination and is limited to only two drug combination	[33]
3	Systems biology	A mathematical and computational model which predicts and helps to understand the network of components and protein or gene targets binding system	Advantages: Helps to identify the key active components by investigating the mechanism of action and aids in the study of synergy of multi-components, prodrugs and novel targets Disadvantages: Requires large data sets	[34-37]
4	PPI network-based models	Based on complex network method it assesses the drug synergy on the basis of network topology relations of target	Inputs - drug targets, protein interactions Outputs - synergistic drug combinations and targets	[38-40]
5	Pathway based models	Based on ordinary differential equations and network motif recognition it identifies the synergy-specific structures of pathway by simulating the dynamic changes	Input - drug targets and interactions. Pathway structures and dynamic changes of pathway components Output - dose response assessment and synergy specific network motif	[41,42]
6	Drug similarity-based models	Similarity calculation, feature selection, classification model for construction of classification model	Input - drug properties Output - distinctive features and synergistic drug combinations	[43,44]
7	Omic based models	Based on reverse engineering of biological networks, omic data and classification model calculates drug association and builds synergy dependent pathway	Input - omic data drug responses Output - synergistic drug combinations and biological networks	[45-47]

PPI: Protein-protein interaction, CI: Confidence interval

Table 8: Evaluation parameters of protein-protein interaction model

Serial number	Name	Basis	References
1	Topology score	Centrality of drug targets aids in the estimation of the synergy mechanism	[48]
2	Topology score combined with other scores. Example: TS+AS	Adjusts the topology-based scores	[48]
3	TSDS	Based on several features like synergistic effect, global effect, node reachability etc., for evaluation of multitargeted combination	[49]
4	Drug combo ranker	Evaluation tool covering aspects based on drug behavior	[50]

TS: Topology score, AS: Agent score, TSDS: Topological score of drug synergy

Advantages

- PPI model is a very forthright and efficient method.
- Helps to disclose the mechanism of synergy by mapping the drug targets onto the disease specific molecular networks along with the attainment of synergistic effect in different drug blends.

Disadvantages

Since the drug behavior is based on several factors such as side effects, pharmacokinetic properties, and drug resistance apart from the drug targets; thus, this method has its limitation and there arises a requirement of more advanced methods which takes into consideration the complex interactions leading to synergy.

Pathway-Based Models

Inside a cell a sequence of chemical reactions that produce a specific response is denoted as a pathway. Therefore, we may elucidate the synergy mechanisms by demonstrating the dynamic changes along with the network structures of pathway.

Dynamic pathway simulation

Dynamic simulation pathway models are denoted in form of network which aids in the study of the dynamic behavior of the drug thus comprehending the mechanism of synergy. From the dose response data compiled from the study, the outcomes are estimated using Loewe additivity and Bliss independence like traditional methods. The dynamic responses are quantified through the ordinary differential equations of the classical chemical kinetics and its alterations to progress reproducing cellular dynamics [Table 9].

Advantages

- Predictive effective combination of drugs
- Demonstrates the mechanism of synergy at pathway level.

Disadvantages

- These models are convoluted and inadequate.

Synergy-Specific Network Motifs in Pathway

To decrease the complexity of the pathway-based models abstract network models using network motifs may be utilized which may depict the key dynamical properties of network. Based on specific focuses different motifs may be generated in different studies [Table 10].

Advantages

This method offers guidance for the rational design of possibly efficient combination of drugs.

Disadvantages

It has several limitations thus there arises a need for more comprehensive network models which takes into consideration drug behavior along with the drug targeting pathways.

Computational Models

In computational models various machine learning methods may be applied for combination of drugs which may be described using multiple drug

Table 9: Different types of pathway-based models

Serial number	Model name	Used in study of	References
1	Kinetic ordinary differential equation model	Based on EGFR model the outcome of EGFR combinatorial kinase inhibitor was assessed and it was observed that the simultaneous inhibition of multiple nodes in signaling cascade with small molecular kinase inhibitor signifying a new promising blend for the treatment of cancer	[52]
2	Modification of classical kinetic ordinary differential equation model	A nonlinear ODE model was used to capture epistasis and saturation effect were used in the combinatorial disconcertion in breast cells A nonlinear ODE model simulate the dynamic changes of signaling pathways in dedifferentiated liposarcoma cells along with experimental drug combination screening used to elucidate synergy of CDK4 and IGFIR inhibition which may rely on AKT pathway	[53,54]
3	Modeling frame based on modular response analysis	Was used in the EGFR signaling pathway and it was observed that if an appropriate downstream inhibitor is combined than the upstream drug treatment are not essentially annulled by downstream mutations	[55]
4	Mathematical model	In the biological pathways the serial and the parallel pathways are simplified	[56]
5	Network based modeling based on the Ingenuity pathway analysis software	To comprehend MI219 and oxaliplatin combination's central mechanism of synergy to understand central synergy mechanisms for the combination of MI219 and oxaliplatin	[57]

ODE: Ordinary differential equation, EGFR: Epidermal growth factor receptor, IGFIR: Insulin-like growth factor-1 receptor, CDK: Cyclin-dependent kinase

Table 10: Synergy-specific network motifs in pathway

Serial number	Model name	Observation	References
1	Ten three- node small network motifs (positive feedback loop, negative feedback loop, positive auto-regulation and feed forward loop)	When targeting on a negative or mutual inhibition loop motif the combinations were observed to be more synergistic	[58]
2	Three-node enzymatic motifs	Synergistic motifs-serial and parallel or mix type structure. Antagonistic motifs-positive feedback loop and downstream link	[59]

properties to build a classification model that may predict or classify the synergistic blends of drug.

Advantages

Computational models help to calculate the combination of drugs.

The mechanism of synergy can be precisely elucidated using informative features.

Disadvantages

Effective features should be designed so as to identify the combination of drugs encompassing confirmed synergistic effect or not.

Identifying active chemical constituents contributing to synergistic interactions

Replacement methodology using grouping of combination index along with fractionation technique are used to identify the active chemical constituents contributing to synergy. The scheme of identification may be understood from the following flowchart of study conducted by Xu *et al*.[66] on *Astragalus membranaceus* and *Paeonia lactiflora*.

These techniques proficiently help in the isolation of active chemical constituents thus forming a base for the mechanistic study of synergism in phytomedicines. Nevertheless, as the inactive fractions are casted off so thus remained untested for their effects as the crude extract had far more superior effect as compared to the active fractions alone[67] [Figure 2 and Table 11].

Advancements for synergy research with the "omic"-technologies

With the advent of the, "omic" technologies a new viewpoint is generated in vindicating the synergistic effects and therefore utilizing them to form an auxiliary phytopharmaceutical generation.[68] Omic technology asses the single and multi-component extracts of plants and surveys the likelihoods of standardization thus, the study of toxicity and safety. It's used in instituting

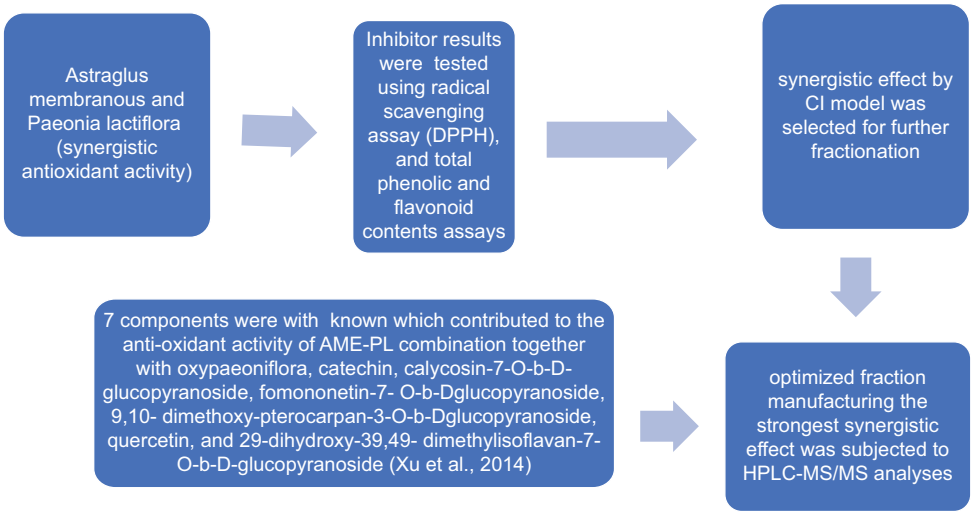

Figure 2: Flow chart representing identification of active chemical constituents contributing to synergistic interactions[66]

Table 11: Synergy in herbal medicines

Herb A	Herb B (API or drugs)	Synergistic effect	Auxiliary information	References
Berberine (RC)	ER antagonists	The combination provided synergistic growth inhibitory effect on MCF-7 cells	Molecular mechanism: Berberine is down regulated, EGFR, HER2, Bcl-2 and COX-2, whereas upregulated IFN-b and p21 Type-pharmacokinetic potentiation by facilitation of actions on various targets and pathways	[60]
Bisbenzylisoquinoli -ne	Vinblastine, paclitaxel and depsipeptide	In SW620 and Ad20 cells the bisbenzylisoquinoline reverses the MDR	ABC transporters Pgp and MRP1 are inhibited by bisbenzylisoquinoline Type-pharmacokinetic potentiation due to MDR elimination	[61]
Baicalein	Gentamicin	Used in vancomycin resistant enterococcus	Method of stufy: MIC Synergy at specific dosages (NA)	[62]
Mentha piperita L.	*Salvia officinalis* L.	Used as anti - caner agent	At 31.25, 62.5, and 125 µg/mL dosage levels, cancer cells treated with *Mentha piperita* L. plus *Salvia officinalis* L. combinations (1:1) showed significantly lower viability than calculated values based on individual extracts [CI=0.67±0.09 (<1)]	
P. polyphylla	5-fluorouracil and oxaliplatin	*P. polyphylla* exerts a synergistic antiproliferative effect by a combination with 5-fluorouracil and oxaliplatin	*P. polyphylla* caused S phase cell cycle arrest, the activation of pro-caspase 3, upregulation of Bax and down-regulation of Bcl-2 proteins expression. Combination of *P. polyphylla* and 5-fluorouracil or Oxaliplatin suppressed the mRNA levels of thymidylate synthase and human excision repair cross-complementing Pharmacokinetics potentiation because of facilitating actions on different targets and pathways	[63]
Green tea	Penicillin G	A combination of green tea and penicillin G showed a noticeable increase of 28.7 mm inhibition zone diameter		[64]
Oxyresveratrol (*A. lakoocha*)	Acyclovir	Synergistic antivirus effects as tested in Vero cells infected with herpes simplex virus	Oxyresveratrol exhibited the inhibitory activity at the early and late phase of viral replication and inhibited the inhibited late protein synthesis Pharmacodynamic synergy because facilitating actions on the same target	[65]

MIC: Minimal inhibitory concentration, MRP: Multidrug resistance proteins, *NA: Not available, RC:* Rhizoma coptidis, EGFR: Epidermal growth factor receptor, API: Ayurvedic Pharmacopoeia of India, *P. polyphylla*: *Paris polyphylla*, *A. lakoocha*: *Artocarpus lakoocha*

communications amongst genes, small molecules, diseases along with pathological process related molecules. It can also be used in the development of individualized medicine, combination therapies of phytomedicines and synthetic pharmaceutically active agents to enhance the therapeutic efficiency and diminish the adverse reactions and to prove them with clinical studies. The Omic technology

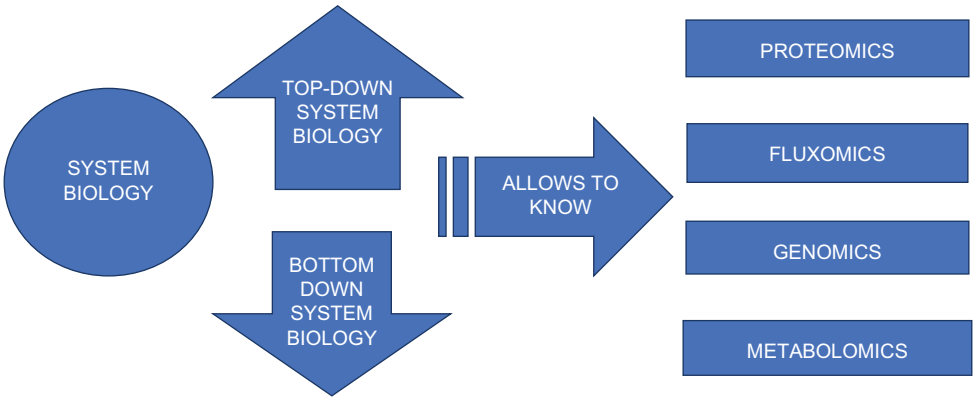

Figure 3: Omic approaches in development of natural products through system biology[67]

along with quantifying biological molecules has also been useful in the prediction of the response of human body to the synergistic drug blends through computational approaches that are specific so as to form new molecular networks.

The omic approach has brought advancements in the arena of systems biology yet it is a complex method and a great challenge despite its reliable outcomes in various high-throughput data such as genomics, genetic science, transcriptomics, and metabolomics which also helps in determining the mechanism of synergy [Figure 3].

The subsequent aspects are of interest group to phytopharmaceuticals:

1. Standardization of single and multicomponent plant extracts
2. Pharmacology and bioavailability of plant extracts and their combinations
3. Prediction and assessment of the toxicity and safety of plant extracts
4. Individualized medicine
5. Synergism, signal cascades, and trigger points of the metabolism
6. Improvement preparations through new combinations of plant components involving ancient systems of medicine and thereby constituting the development of a replacement generation of standardized phytopharmaceuticals.

CONCLUSION

In the herbal medicines, the mechanism of synergy may be ascertained in forming novel and innovative multi-targeted drugs and combination of drugs which may show superior therapeutic effect when used in combination. The mechanism of synergy consists of numerous types of interactions such as with multiple sites, targets and pathways guided by scheduling, environmental, genetic, and behavioral profiles. A phytotherapy that comprises of a combination phyto leads along with the components of inferior potencies may form appropriate combinations on a personalized basis to accomplish satisfactory level of efficiency and safety.

Synergy is a marvel in field of pharmacognosy which inspires that the plant extracts are much more just the isolated components and have much more to offer with the help of study of clinical bioequivalence the phyto components along with the synthetic drugs may enhance the therapeutic efficacy. However, this synergistic screening study is very complex and demanding both technically and methodically yet from future perspective its likely to advance pharmacotherapy significantly.

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