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Network Ethnopharmacology Approaches for Formulation discovery

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Lifestyle disorders like obesity, diabetes, cardiovascular diseases, and cancers are difficult to manage using one drug-one target approach and so require a multi-targeted approach. Any drug-whether chemical, botanical or biological-will have inherent limitations if it is focused only on a single target when dealing with polygenic syndromes. A new branch known as network pharmacology which integrates systems biology and computational biology has emerged to study drug interactions with multiple targets. Several traditional multi-botanical formulations are widely used globally; however, their rationale and scientific evidence for pharmacodynamic actions remain insufficient. A systematic study of network ethnopharmacology-network pharmacology of medicinal botanicals- is considered as promising approach to understand the scientific basis of intelligent formulations which would facilitate transition from single target based drug discovery to multi-target based rational formulation discovery.This article briefly describesnetwork pharmacology and demonstrates the ***Rasayana*** property of ***Triphala***in the light of this emerging technique.

Keywords: Ayurveda, Bioactives, Ethnopharmacology, Network pharmacology, *Triphala*

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The scientific interest in natural products as drugs has been continuing especially since Galen era. In several traditional systems, medicinal botanicals (plants) have been in use for therapeutic purposes. Typically, pharmacognosy is the study of crude drugs but the new pharmacology has emerged as an interdisciplinary field of research[1]. The advances in synthetic chemistry coupled with pharmacology have led to many new chemical entities as drugs. This resulted in a little lull period for natural product research. However, in recent years, the natural product drug discovery is reemerging mainly due to their superior chemical diversity and safety over synthetic compound libraries.It is estimated that over one hundred new, natural product-based leads are in clinical development[2]. About 60% of anticancer and 75% of anti-infective drugs approved from 1981 to 2002 have natural origins[3]. Many active compounds (bioactives) from traditional medicine sources could serve as good starting compounds and scaffolds for rational drug design.This indicates the need to rediscover the drug discovery process and multi-target formulation discovery is seen as future in this direction[4]. Many experts feel that it would be cheaper and more efficient to study botanicals described in ancient texts[5]. In the past, impressive successes with botanical bioactives, most notably, quinghaosu and artemisinin from Traditional Chinese Medicine (TCM) have been reported. Numerous bioactive molecules have also come out of the Ayurvedic experiential base, examples include *Rauwolfia* alkaloids for treating hypertension, *Psoralens* for vitiligo, *Holarrhena* alkaloids for amoebiasis, guggulsterons as hypolipidemic agents, *Mucunapruriens* for Parkinson's disease, piperidines as bioavailability enhancers, baccosides in mental retention, picrosides for hepatic protection, phyllanthins as antivirals, curcumin for inflammation, glycosidesa withanolides and many other steroidal lactones as immunomodulators[6]. The transition of research from synthetic compounds to natural compounds is showing the need to evolve new techniques in the discovery processes of natural products. Normally drug discovery follows the one drug-one target-one disease approach. However, advances in 'omics' techniques point out the limitations of this approach. Today, we are dealing with polygenic syndromes and not just isolated diseases, hence multi-target approaches are

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necessary[7]. Lifestyle disorders like obesity, diabetes, cardiovascular diseases and cancers can be well managed with a multi-targeted approach. Many bioactives from traditional medicine sources can deal with multiple targets simultaneously and may have synergistic effects. Understanding the molecular mechanisms of actions of bioactives would pave the way for better exploration of natural medicine, which will help to rediscover the traditional wisdom.

Network pharmacology

Recently, a new technique called network pharmacology has emerged which attempts to understand drug action and interactions with multiple targets[8]. It uses computational power to systematically catalogue the molecular interactions of a drug molecule in a living cell. Network pharmacology emerged as an important tool in understanding the relationship between drug action and disease susceptibility genes[9]. It also attempts repurposing existing drug molecules for different therapeutic indications. Integrating systems biology and network pharmacology can accelerate the search for druggable targets and help in designing new drugs, which modulate multiple biological targets[10,11]. Network pharmacology opens up new therapeutic options as well as aid to improve safety and efficacy of existing medications. Chinese Scientists have been employing this technique to better understand and explore Traditional Chinese Medicine (TCM). Li Shao[12] pioneered in using network pharmacology in TCM to understand ZHENG (syndrome of TCM) oriented effects and botanical synergism. The potential of TCM formulations as multiple compound drug candidates has been studied using TCM based network pharmacology[13]. Construction of a database containing 19,7201 natural product structures followed by their docking to 332 target proteins of FDA approved drugs has showed the amount of space shared in the chemical space between natural products and FDA drugs[14]. Molecular docking technique plays a major role in network pharmacology. The possible interactions of bioactives with molecular targets can be analyzed using this technique. Molecular docking based network pharmacology can be a useful tool to computationally elucidate the combinatorial effects of traditional medicine to intervene disease networks[15]. An approach that combines network pharmacology and pharmacokinetics has been proposed to study the material basis of TCM formulations[16]. These approaches can be extrapolated to explore other traditional medicine formulations too.

Recently, we demonstrated how the technique of network pharmacology could be used to study Ayurvedic formulations[17,18]. The bioactive-target-disease network in the study showed the potential of *Triphala* in multiple disease management through multi bioactive-multi target interactions. *Triphala* is one of the most popular and widely used Ayurvedic formulations. *Triphala* contains pitted fruits of three myrobalans–*Emblicaofficinalis* Gaertn (EO; *Amalaki*) also known as *Phyllanthusemblica*, *Terminalia bellerica* (Gaertn.) Roxb (TB; *Vibhitaka*) and *Terminalia chebula* Retz (TC; *Haritaki*). *Triphala* is the drug of choice for the treatment of several diseases, especially those of metabolism, dental and skin conditions, and treatment of cancer[19]. It has a very good effect on the health of heart, skin, eyes and helps delaying degenerative changes, such as cataracts[20]. According to Ayurveda, *Triphala* is an effective medicine to balance all three *Dosha-Vata*, *Pitta* and *Kapha*. It is considered as a good rejuvenator *Rasayana*, which facilitates nourishment to all tissues or *Dhatu*. Here, we elucidate the *Rasayana* property of *Triphala* by generating ethnopharmacological networks. The networks give the picture of the complex interactions of bioactives with molecular targets expressed in different tissues of the body. We refer the pharmacological network of botanicals as 'ethnopharmacological network' and the technique as 'network ethnopharmacology'.

Methods

Knowledge bases for network ethnopharmacology

For developing bioactive-targetnetworks, we used the methodology as described in our earlier reports[17,18]. In brief, the information regarding the bioactives of the three myrobalans- EO, TB and TC-were retrieved from the Universal Natural Products Database (UNPD). UNPD is designed to be a comprehensive resource of natural products for virtual screening. At present, it comprises of 229,358 structures[21]. The '.sdf' files having the structures of the bioactives were searched for identifying their targets using special tool 'find my compounds' target' in Binding database or 'Binding DB'[22]. This searches the exact or similar compounds in the database and retrieves the target information of those compounds. The similarity search gives the structurally similar

compounds with respect to the degree of similarity as scores to the queried structure, where 1 is the highest possible value. A score of 1 indicates that either the exact queried compound is present or it gives a 100% structural similarity to another compound in the database. The targets of those bioactives having a score equal to 1 were selected for this study to improve the accuracy of results. Binding DB is connected to numerous other databases, which were used to extract more information regarding the targets. The protein symbols were taken from UniProt[23] using the UniProt IDs given in Binding DB. The information regarding tissue protein expression and protein class were gathered from 'The Human Protein Atlas'(HPA) database[24]. HPA is an open database showing the spatial distribution of proteins in 44 different normal human tissues. The database also gives information regarding sub-cellular localization and protein class.

Network construction

A network is the schematic representation of the interaction among various entities called nodes. These nodes are connected by lines termed edges. The nodes of the networks in the current study include: ***Triphala*** and its constituent botanicals (EO, TB and TC), the bioactive compounds present in the constituent botanicals, the targets of the bioactives, the corresponding tissues in which the targets are expressed, the tissue types in which the tissues belong and the target class. The networks were constructed using Cytoscape 3.2.1, a java based open source software platform for visualizing complex networks and integrating them with any type of attribute data[25]. The bioactive-target-tissue networkshows the connection of bioactive to the tissue of action through targets.The bioactive-target-target class gives the information of the target classes to which the ***Triphala*** bioactives are interacting. The counts of nodes -such as bioactives, targets- can vary based on the knowledge bases which are relied on for data collection and the timeframe of data collection. This change is due to the databases being under periodical updates. For this study, we used the data collected during May 2015.

Results

***Triphala* with reference to *Rasayana* property**

Triphala contains 174 bioactives based on existing literature[17]. The molecular targets of those bioactives which gave Score 1 results in Binding DB were taken in the study. Forty of such bioactives are interacting with 44 human protein targets. The Score 1 bioactives of ***Triphala*** bioactives are given in Table 1. The expression profiles of 39 of those targets were available in HPA. The bioactive-target-tissue network of ***Triphala*** shows the wide range simultaneous action of its bioactives in 45 different tissues, which cover the 11 physiological systems, through 39 targets (Fig. 1). The network analysis shows that, ***Triphala*** bioactives are regulating the blood and immune system, and endocrine system through 34 targets; cardiovascular system through 21 targets; central nervous system through 33 targets and digestive system through 38 targets.The male and female system through 35 and 37 targets respectively.Liver and pancreas through 35, the respiratory system through 37, skin and soft tissues through 34 and

Table 1—Score 1 bioactives of ***Triphala*** botanicals

E. officinalis	*T. bellerica*
1,2,3,4,6-Pentagalloylglucose	1,2,3,4,6-Pentagalloylglucose
1,2,3,6-Tetrakis(O-Galloyl)-Beta-D-Glucose	Chebulanin
1,2,3-Benzenetriol	Chebulinic Acid
2-sitosterol	Ellagic Acid
3-Galloylgallic Acid	Gallic Acid
Catechin	Tannic Acid
Chebulanin	
Ellagic Acid	
Epicatechin	*T. chebula*
Epicatechin-(4beta->8)-Epigallocatechin 3-O-Gallate	1,2,3,4,6-Pentagalloylglucose
Epicatechin-(4beta-8)-Gallocatechin	3,4,5-Trihydroxybenzenoic Acid
Epigallocatechin 3-Gallate	Casuarinin
Gallocatechin	Chebulanin
Gallussaeure	Chebulinic Acid
Kaempferol	Corosolic Acid
Lauric Acid	Ellagic Acid
Mucic Acid	Maslinic Acid
Naringenin	Punicalagin
Nonadecylic Acid	Punicalins
Prodelphinidin A-1	Sennoside A
Prodelphinidin B1	Tannic Acid
Prodelphinidin B2	Terflavin A
Prodelphinidin B-2'	Terflavin B
Quercetin	Terflavin C
Tannic Acid	Trans-3,3',4',5,7-Pentahydroxylflavane
Tercatain	
Vanillic Acid	

urinary tract through 35 protein targets. The detailed list is given in Table 2.The potential of the bioactives to modulate multiple targets which are expressing in a wide range of tissues that almost cover the entire body systems explains the ***Rasayana***, nourishment of all tissues or ***Dhatu***, property of ***Triphala***.

The forty-four target proteins of ***Triphala***fall under 20 classes according to HPA classification. Based on this information a ***Triphala*** 'bioactive-target-target class' network was constructed (Fig. 2). Twenty-seven targets of ***Triphala*** bioactives are enzymes, 26 are plasma proteins, 19 are cancer-related proteins and 17 are other disease related proteins. Fifteen targets which are FDA approved drug targets include tyrosine-protein kinase receptor UFO (AXL), carbonic anhydrase II (CA2), carbonic anhydrase VII (CA7), plasminogen activator inhibitor 1 (SERPINE1), proto-oncogene tyrosine-protein kinase Src (SRC), tyrosine-protein kinase TIE-2 (TIE2)and vascular endothelial growth factor 2 (VEGFR2). Six proteins that come under potential drug targets are tissue-nonspecific alkaline phosphatase precursor (ALPL), AXL, cytochrome P450 1A1 (CYP1A1), Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), hepatocyte growth factor receptor (HGF) and telomerase reverse transcriptase (TERT). The list of FDA approved and potential drug targets which can be modulated by ***Triphala***bioactives are given in Table 3. ***Triphala*** bioactives are also shown to target six RAS related proteins such as HGF, IGF1R, insulin receptor (INSR), placenta growth factor (PGF), TIE2 and VEGFR2. Three ***Triphala*** targets- aryl hydrocarbon receptor precursor (AHR), mothers against decapentaplegic homolog 3 (SMAD3) and transcription Factor STAT1 (STAT1) - are transcription factors. The ***Triphala*** 'bioactive-target-target class 'network exposes ways to explore new

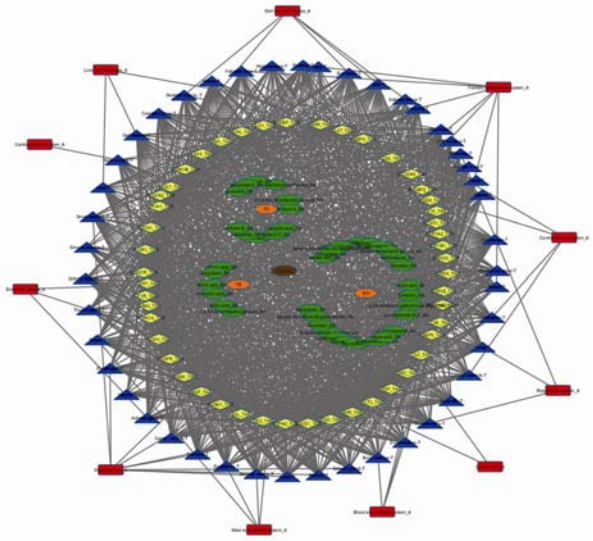

Fig. 1—Ethnopharmacology network of 'bioactive-target-tissue-tissue type' of ***Triphala***. This network connects myrobalans (orange hexagons), bioactives (green ellipses), targets (yellow diamonds), tissues (blue triangles) and tissue types (red rectangles).

Table 2—Tissue distribution of ***Triphala*** targets

Tissue Type	Number of Targets	Examples of Targets
Blood and immune system	34	FLT3, PGD, RGS4, SRC
Cardiovascular system	21	FLT3, IGF1R, TIE2, AXL
Central nervous system	33	NUAK1, PGF, PTPN2, STAT1
Digestive tract	38	CA7, DYRK1A, FABP5, FLT3
Endocrine glands	34	ALDR1, ALPL, BACE1, NOX4
Female reproductive system	37	CYP1A1, DYRK1A, FABP5, PLAT
Liver and Pancreas	35	GSTA1, HGF, HMGCR, IGF1R
Male reproductive system	35	GLO1, GSTA1, PGD, PGF
Respiratory system	37	FABP5, FLT3, RGS4, SERPINE1
Skin and soft tissues	34	CA14, NOX4, PTPN2, TIE2
Urinary tract	35	HGF, IGF1R, RGS4, VEGFR

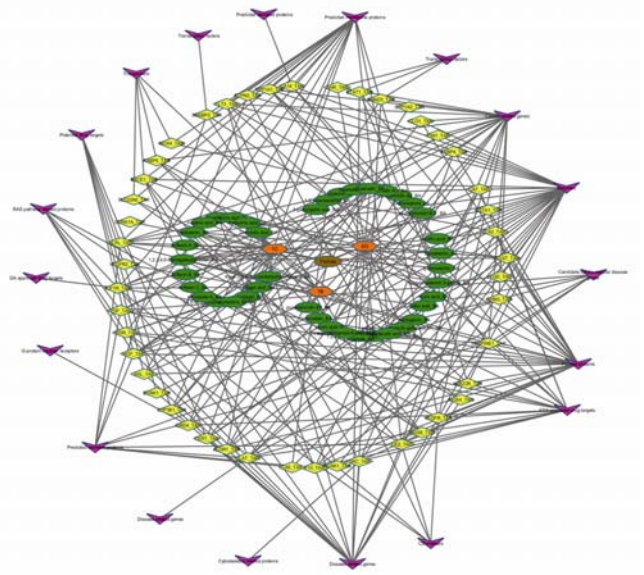

Fig. 2—Ethnopharmacology network of 'bioactive-target-target class' of ***Triphala***. This network connects myrobalans (orange hexagons), bioactives (green ellipses), targets (yellow diamonds) and target class (pink Vs).

Table 3—Targets of *Triphala* bioactivesthat come under the class FDA approved and potential drug targets

Protein class	*Triphala* targets
FDA approved targets	Tyrosine-protein kinase receptor UFO (AXL)
	Carbonic anhydrase II (CA2)
	Carbonic anhydrase VII (CA7)
	Plasminogen activator inhibitor 1 (SERPINE1)
	Proto-oncogene tyrosine-protein kinase Src (SRC)
	Tyrosine-protein kinase TIE-2 (TIE2)
	Vascular endothelial growth factor 2 (VEGFR2)
	Factor 10 (F10)
	Tyrosine-protein kinase receptor FLT3 (FLT3)
	HMG-CoA reductase (HMGCR)
	Insulin receptor (INSR)
	Placenta growth factor (PGF)
	Tissue-type plasminogen activator (PLAT)
	Testis-specific androgen-binding protein (SHBG)
	Solute carrier family 22 member 6 (SLC22A6)
Potential drug targets	Tissue-nonspecific alkaline phosphatase precursor (ALPL)
	Tyrosine-protein kinase receptor UFO (AXL)
	Cytochrome P450 1A1 (CYP1A1)
	Dual specificity tyrosine-phosphorylation-regulated kinase 1 A(DYRK1A)
	Hepatocyte growth factor receptor (HGF)
	Telomerase reverse transcriptase (TERT)

bioactive leads for potential targets as well as gives insight into the need for multi-targeting.

Discussion

Network pharmacology is a powerful tool to study and analyze the vast accumulated data generated as a result of the advancements in 'omics' technologies. The emergence of network pharmacology heads for rethinking the paradigm shift from the conventional one drug-one target-one disease approach to multi-compound – multi-target approach to deal with multifactorial diseases[26]. This also exposes ways to rediscover traditional medicine wisdom. Also, network pharmacology gives the picture of wide range effects and side effects of drugs[27,28]. In this study example, we have taken the data collected during May 2015 which showed differences in number of entities such as Score 1 bioactives and targets from our previous report. Though the data is inconclusive as it is based on existing information and we relied on single database each for bioactive and target identification, it satisfactorily explains the *Rasayana* property of *Triphala* by depicting the interaction potential towards multiple targets that cover almost all tissues of the body. *Triphala* bioactives target 39 proteins which are expressed in 45 different tissues that come under 11 tissue types. The understanding of synchronized modulation of multiple targets would help to reveal the intelligence behind Ayurvedic formulations. In cancer research, a number of natural products have been explored for anticancer potential. Natural products are gaining attraction in anti-cancer research as they show a favorable profile in terms of absorption and metabolism in the body with low toxicity. A study which used molecular docking to check the possible interactions between all known bioactives and cancer targets showed the multi-target interactions of bioactives[29]. This gives insight to explore molecular pharmacology of botanical bioatives to better understand the multiple target modulation mechanism. Bioactive-target-target class network of *Triphala* showed the involvement of 19 cancer-related targets. Further experimental studies in this line to uncover the modulation mechanism may lead to the development of novel bioactive formulations for cancer. Many of the TCM formulations which have been studied using network pharmacology revealed the possible mechanism of action as well as uncovered novel drugs and targets[30,31,32,33]. Network pharmacology coupled to sophisticated spectroscopical analysis like ultra-performance liquid chromatography-electrospray ionization-tandem mass spectroscopy is a useful approach to study the absolute molecular mechanism of action of botanical formulations[34]. Bioactive-target analysis have shown that some of the botanical formulations are more effective than their corresponding market drug-target interactions[35]. This indicates the potential of network pharmacology to better understand the potency of botanical formulations and to develop efficient and economic treatment options. The holistic approach of botanical formulations can be better explained by network ethnopharmacology. A recent study reported this property by exemplifying a TCM formulation against viral infectious disease[36]. The study found that the formulation acts in a distinctive way that not only target the proteins in the viral infection cycle but regulate the proteins of host defense system. This unique property provides the broad and non-specific anti-pathogenic activity to botanical formulations. Network pharmacology also serves to

document and analyze the clinical prescriptions of traditional medicine practitioners[37]. A traditional medicine network which links bioactives to clinical symptoms through targets and diseases is a novel way to explore the basic principles of traditional medicines such as ZHENG in TCM[38], and the same can be applied to understand '*Prakriti*'[39] oriented practice in Ayurveda. In summary, network ethnopharmacology is a useful technique to scientifically explore the traditional knowledge as well as to improve the existing drug discovery approach. This has relevance in the current scenario where the world is looking for natural alternatives over synthetic drugs. The ethnopharmacological networks help to understand the mechanism of action of formulations which can be further explored to identify novel therapeutic leads and targets, and to develop new formulations.

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