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ORIGINAL ARTICLE

Network Ethnopharmacological Evaluation of Ashwagandha (*Withania somnifera*) For Breast Cancer Activity against Its Potential Targets

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ABSTRACT

Up to the year 2030, nearly 10 million new cases of breast cancer would have been discovered, making it the most prevalent disease in the world. Breast cancer may be fought using natural chemicals, which can block malignant cell growth, and regulate cancer-related processes. Ayurveda also has an abundant resource of botanical products containing diverse pharmacoactive ingredients and millennia of experience of clinical applications for health benefits. But there is a lack of evidence-based research to demonstrate its efficacy and potential. The potential of Ayurvedic medicine needs to be explored further with modern scientific validation approaches for better therapeutic leads. A significant "rasayana herb" in Ayurveda, Ashwagandha demonstrates a wide range of traditional and pharmacological potential. It is crucial in the fight against many types of cancer. In current study we have aimed to predict bioactives from Ashwagandha acting against potential targets of breast cancer for its cure using network ethnopharmacological approach. Information for the network was gathered from the following databases: Dr. Duke's, IMPPAT, PubChem, Binding DB, UniProt, and DisGeNET. Cytoscape 3.10.0 (Jave 17.0.5) was used in the creation of the network. Screened bioactives having similarity index more than 0.6 acting against potential targets were examined. Study revealed that out of 52 phytoconstituents 32, 2, 2, 13, and 2 bioactive compounds are acting against progesterone, estrogen, estrogen beta, hypoxia-inducible factor, and epidermal growth factor receptor, respectively. The top most bioactive compound was studied for its ADMET properties to established drug suitability. The ADMET properties were estimated using ADMETlab 2.0.

Keywords: *Breast Cancer, Ashwagandha, Potential Targets, Network Ethnopharmacology, Ayurveda.*

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INTRODUCTION

The second-leading cause of cancer-related death in women is breast cancer, which is the cancer wherein women are most frequently diagnosed. Evidence from the literature has revealed that recent and ongoing research has a significant impact on enhancing the clinical prognosis for breast cancer. This has been linked to advancements in the management of breast cancer in the areas of screening, diagnosis, and therapeutic approaches. However, triple negative breast cancer's poor prognosis and medication resistance provide significant obstacles that are also today's difficulties for disease control. Similar to this, the rising incidence and fatality rates from breast cancer in the underdeveloped world's population are of particular concern. (1). Breast cancer risk assessment is always changing as new information about the factors that increase and lower breast cancer risk makes it possible to identify high-risk women more effectively and determine which risk-reduction strategies would be most beneficial for them. A chance to reduce the occurrence of breast cancer through evidence-based treatments is provided by the continued development of breast cancer Risk Assessment and Management Programs (2). As an increased target gene of estrogen receptor and an estrogen-dependent gene, the progesterone receptor controls the function of estrogen receptors in breast cancer. Especially in cases of hormone-positive breast cancer,

progesterone receptor is a useful predictive biomarker for the disease. Luminal A cancers, which have a better baseline prognosis than tumours with a worse baseline prognosis, are more likely to exhibit high progesterone receptor expression (ie, luminal B) (3). A vital medicinal herb of the Indian subcontinent, *Withania somnifera* is also known as "Ashwagandha" or "Indian ginseng." Over a period of 3,000 years, Indian Systems of Medicine have routinely employed it to cure a variety of ailments, either by itself or in conjunction with other plants. Ashwagandha is a member of the Withania genus and Solanaceae family. It has a wide range of phytochemicals with a variety of biological effects. Numerous biological effects of Ashwagandha have been proven, including anti-cancer, anti-inflammatory, anti-diabetic, anti-microbial, anti-arthritic, anti-stress/adaptogenic, neuro-protective, cardio-protective, hepato-protective, and immunomodulatory qualities. Additionally, studies on Ashwagandha have shown that it can modulate mitochondrial activity, control apoptosis, reduce reactive oxygen species, and reduce inflammation. It can also improve endothelial function (4). A significant "rasayana herb" in Ayurveda, Ashwagandha demonstrates a wide range of traditional and pharmacological potential. It is crucial in the fight against many types of cancer. The primary ingredients in Ashwagandha that give it its anticancer properties are withanolides. Through a variety of mechanisms, including cell cycle arrest, cell membrane disruption, mitotic catastrophe, chromosomal segregation, and proteasomal degradation, they cause malignant cells to undergo apoptosis (5). Critical analysis of the available material points to the anti-cancer potential of Ashwagandha with a substantial role in cancer prevention. Indicators of apoptosis, proliferation, and metastasis in cancer are regulated in relation to the potential mechanisms driving these effects. Ashwagandha may lessen inflammatory responses as well as the activity of enzymes linked to cancer invasion and metastasis. The properties of Ashwagandha are most likely mediated by withanolides, which may activate tumour suppressor proteins to reduce the proliferation potential of cancer cells. Withanolides also regulate the energy requirements and genetic instability of cancer cells. The reported results indicate that a deeper understanding of the molecular mechanisms by which Ashwagandha suppresses angiogenesis and improves immunosurveillance is required. Additionally, Ashwagandha can increase the efficacy and security of cancer treatments (6). Many in vitro, in vivo, and clinical experiments detailed the use of different Ashwagandha species. They found evidence that ashwagandha prevents and treats breast cancer by having anti-apoptotic, anti-metastatic, anti-invasive, and anti-inflammatory properties (7). Even though there are many indications that Ashwagandha has anti-breast cancer properties, a thorough knowledge of how these bioactives interact with potential targets and how they work synergistically together is yet unknown. Additionally, the exact mechanism through which a given bioactive leads to a therapeutic benefit is still unknown. The main aim of this study is network ethnopharmacological based evaluation of the breast cancer activity of Ashwagandha (*Withania somnifera*) traditionally known for anti-cancerous effect towards progesterone (PGR), estrogen (ESR1), estrogen beta (ESR2), Hypoxia-inducible factor (HIF1A), and epidermal growth factor receptor (EGFR). Thus study involves exploration of molecular mechanism of these potential targets for effective bioactives from Ashwagandha (*Withania somnifera*) in breast cancer treatment using in-silico methods.

MATERIAL AND METHODS

Network Pharmacology Studies

Data mining for Phytochemicals

In this investigation, the phytoconstituents from Ashwagandha that have been traditionally documented to possess anti-breast cancer were employed. Dr. Duke's Phytochemical and Ethnobotanical Databases online platform [8], Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 [9], and literature mining were used to gather information about the phytoconstituents of Ashwagandha.

For the investigation, the '.sdf' formats, which are freely available and available in the 3D structures of phytoconstituents, were utilized. In PubChem [10], look up the common names and precise structures of the phytoconstituents from Ashwagandha.

Establishment of Target

The RCSB PDB database [11] was used to gather data on PGR, ESR1, ESR2, HIF1A, and EGFR. The species are confined to human sources, and target is identified. PGR, ESR1, ESR2, HIF1A, and EGFR as therapeutic targets related to breast cancer was searched by using DisGeNET [12]. Through UniProt [13], standard names for protein targets were found.

Screening of bioactives by polypharmacology

The SDFs containing the structures of phytoconstituents from Ashwagandha were uploaded to the Binding DB (https://www.bindingdb.org) for the purpose of predicting the binding of bioactives to PGR, ESR1, ESR2, HIF1A, and EGFR for the treatment of breast cancer. Those bioactives having a score between 0.6 and 1 has been chosen. The multiple databases that Binding DB is connected to were leveraged to

extract additional data on the targets. The UniProt IDs provided in Binding DB were used to retrieve the protein symbols from UniProt. DisGeNET was searched for associations between the bioactives, targets and breast cancer.

Network Construction

Cytoscape 3.10.0. was used to visually represent the network, analyse, and update the data. The data pairs of Ashwagandha with bioactive PCIDs; bioactive PCIDs with PGR, ESR1, ESR2, HIF1A, and EGFR; and PGR, ESR1, ESR2, HIF1A, and EGFR with breast cancer were built in excel programmed files. The data pairs were imported and created a network map of the therapeutic components and disease targets. The nodes in the network diagram stand in for selected Ashwagandha; bioactives; PGR, ESR1, ESR2, HIF1A, and EGFR; and breast cancer, while the edges show how the nodes are connected. The network was examined using the 'Network Analyzer' function.

ADMET Studies

The computational ADMET predication (absorption, distribution, metabolism, excretion, and toxicity) are constitutive methods used in modern drug discovery to predict the drug pharmacokinetics and toxicity. ADMET properties are necessary for the selection and development of drug candidates. The ADMET properties for top most compounds were estimated using ADMETlab 2.0. Greater HIA denotes that the compound could be better absorbed from the intestinal tract upon oral administration. The penetration through the Blood Brain Barrier (BBB Penetration) came out to be a best drug candidate. However, determining the toxicity of chemical compounds is necessary to identify their harmful effects on humans, animals, plants or the environment. Here we have used the ADMETlab 2.0 (https://admetmesh.scbdd.com/) to check the prediction of ADMET properties.

RESULTS AND DISCUSSION

Network Pharmacology Studies

The number of bioactives from Ashwagandha (*Withania somnifera*) having activity against selected potential targets, equal to or greater than 0.6 similarity index (scoring bioactives) were found to be 34 with network ethanopharmacology approach. The number of bioactives having interaction with PGR was 32, with ESR1 was 2, with ESR2 was 2, with HIF1A was 13 and with EGFR was 2 as shown in **Figure 1.** The screened bioactives from Ashwagandha using polypharmacology approach was subjected for network construction and analysis using Cytoscape v. 3.2.1 software. Analysis of the nodes and edges of the networks is given in **Table 1.** The nodes of networks representing Ashwagandha and their bioactives, targets of bioactives and the diseases associated with the targets are noted in the Table 1. The table also gives bioactives of Ashwagandha that have maximum interactions with targets. Network showed that it contains 34 high scoring bioactives, which are involved in breast cancer through 5 targets. The Ashwagandha bioactives with highest number of interactions include PCID222284, PCID14106343, results are given in **Table 2**.

Table 1: Nodes and edges of Ashwagandha (*Withania somnifera*) network

Parameter	Ashwagandha (*Withania somnifera*)
Bioactives	34
High scoring bioactives	1 (Beta-sitosterol)
Targets	5
Diseases	1
Bioactive-target interactions	47
Highly interacting bioactives	2 (PCID222284, PCID14106343)

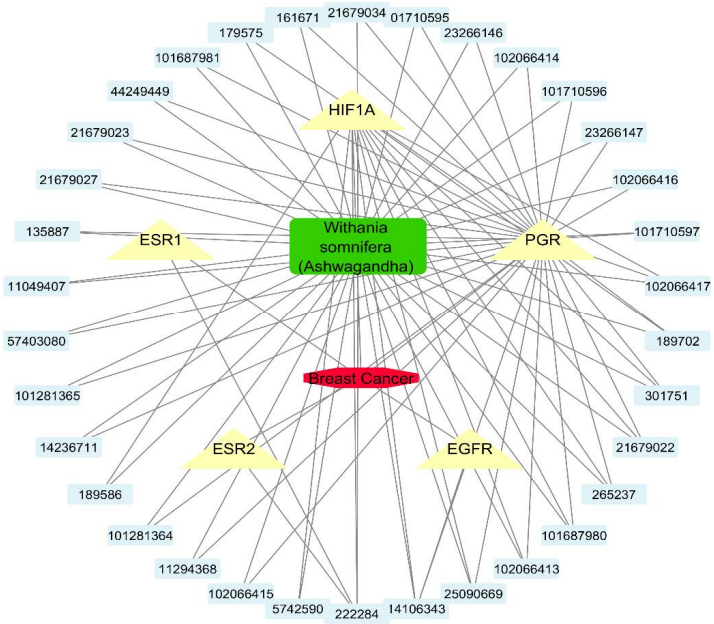

Figure 1: Pharmacology Network of Ashwagandha (green rectangle) which connect bioactives (blue squares) with targets (yellow triangles) and diseases (red hexagon)

Table 2: Targets and bioactives interactions

Targets	Bioactives
PGR	32
ESR1	2
ESR2	2
HIF1A	13
EGFR	2

ADMET Studies

Beta-sitosterol (PCID222284) showed average oral absorption rate, high gastrointestinal absorption, no blood-brain permeability, and very lower skin permeation. Absorption properties except Pgp-inhibitor (median) showed excellent results. Molecule showed less BBB penetration and excellent volume of distribution. Both absorption and distribution results indicate compound to be good candidate for oral administration. In case of metabolism, the molecule was found to be a p-glycoprotein positive substrate; however, the molecule did not served as a substrate for CYP2C19, CYP2C9, CYP2D6 and CYP3A4. From the predicted pharmacokinetic parameters, the observed values of the Beta-sitosterol lay within the prescribed limits as given in **Table 3**.

Table 3: In silico ADMET studies data of Beta-sitosterol.

Category	Model	Points
Absorption	Caco-2 Permeability	-4.756
	MDCKPermeability **TheMadin-Darby canine kidney:**	8.6e-06
	Pgp-inhibitor(Permeabilityglycoprotein)	**-**
	Pgpsubstrate(permeability glycoprotein)	**---**
	HIA (Human Intestinal Absorption)	---
Distribution	PPB(Plasma protein binding)	98.314%
	VD(Volume Distribution)	1.963
	BBB Penetration(Blood Brain Barrier)	++
	Fu	1.485%
Metabolism	CYP1A2inhibitor (Cytochrome P4501)	---
	CYP1A2 substrate	**-**
	CYP2C19 inhibitor(Cytochrome P4502)	**---**
	CYP2C19 substrate	**+++**
	CYP2C9 inhibitor(Cytochrome P450 family 2 subfamily C member 9)	---

	CYP2C9 substrate	-
	CYP2D6 inhibitor(Cytochrome P450 2D6)	---
	CYP2D6 substrate	-
	CYP3A4 inhibitor(Cytochrome P450 3A4)	--
	CYP3A4 substrate	++
Excretion	CL	16.686
	T1/2(half life of the reaction)	0.013
Toxicity	hERG Blockers (Human ether à-go-go related gene)	---
	H-HT	--
	DILI	--
	AMES Toxicity	---
	Rat Oral Acute Toxicity	---
	FDAMDD	++
	Skin Sensitization	--
	Carcinogencity	---
	Respiratory Toxicity	+
	Bioconcentration Factors	2.963
	IGC50(Inhibition Growth concentration)	4.984
	LC50FM(Lethal Dose)	5.365
	LC50DM	6.231
Physicochemical Property	Molecular Weight (MW)	414.390
	Volume	482.068
	Density	0.860
	nHA(No of hydrogen acceptor)	1
	nHD(No of hydrogen donor)	1
	nRot(No of rotatable bonds)	6
	nRing(No of rings)	4
	MaxRing(Maximum no of rings)	17
	nHet(No of heteroatoms)	1
	fChar(Formal charge)	0
	nRig (No of rigid bonds)	20
	Flexibility	0.300
	Stereo Centers	9
	TPSA(Topological polar surface area)	20.230
	logS(Measuring solubility)	-7.052
	logP(Measuring lipophliicity)	7.663
	logD(Distribution Coeffiecient)	6.329

Note-the prediction probability values are transformed into six symbols: 0-0.1(---), 0.1-0.3(--), 0.3-0.5(-), 0.5-0.7(+), 0.7-0.9(++), and 0.9-1.0(+++)

CONCLUSION

The study elucidated a network ethnopharmacological based of PGR, ESR1, ESR2, HIF1A, and EGFR inhibition by various bioactive phytoconstituents from Ashwagandha (*Withania somnifera*). Experimental validation of the network findings would aid in understanding the rationale behind anti-breast cancer activity as well as aid in bioactive formulation-based drug discovery. It was discovered that beta-sitosterol is a high-scoring molecule that may inhibit up to four targets. The observed values indicated that the compound in the screen is a good drug candidate based on the predicted pharmacokinetic parameters.

CONFLICT OF INTEREST

Declared none

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