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NETWORK PHARMACOLOGY ANALYSIS OF ASHWAGANDHA (WITHANIA SOMNIFERA) AGAINST ANXIETY DISORDERS THROUGH NEUROTRANSMITTER AND NEUROINFLAMMATORY SIGNALING PATHWAYS

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ABSTRACT

Anxiety disorders are among the most prevalent psychiatric disorders worldwide and significantly affect quality of life. Ashwagandha (*Withania somnifera*) is a traditional medicinal herb widely used for stress and anxiety management. However, its underlying molecular mechanisms remain incompletely understood. To investigate the pharmacological mechanisms of Ashwagandha against anxiety disorders using a network pharmacology approach. Bioactive compounds were identified from public databases and literature. Potential targets were predicted using target prediction tools. Anxiety-related genes were collected from disease databases. Common targets were analyzed using protein-protein interaction networks, Gene Ontology enrichment, and KEGG pathway analysis. Key bioactive compounds included withaferin A, withanolide A, and withanone. Hub targets identified were AKT1, TNF, IL6, MAPK1, BDNF, DRD2, and SLC6A4. Enrichment analysis indicated involvement of serotonergic synapse, dopaminergic synapse, PI3K-Akt signaling, MAPK signaling, and neuroinflammatory pathways. Ashwagandha exerts anxiolytic effects through multi-target and multi-pathway mechanisms involving neurotransmitter regulation, neuroprotection, and anti-inflammatory activity.

KEYWORDS: Ashwagandha, Anxiety, Network Pharmacology, Withania somnifera, Neurotransmitters, PI3K-Akt.

1. INTRODUCTION

Approximately 301 million individuals globally suffer from anxiety disorders, which are a major public health issue. Medications such as benzodiazepines and selective serotonin reuptake inhibitors are commonly used to treat anxiety. However, they can produce undesirable side effects of sedation and dependence, in addition to impairing cognitive function.

Ashwagandha (Withania somnifera) is a significant medicinal herb in traditional Ayurvedic medicine. It exhibits adaptogenic, antioxidant, anti-inflammatory, and neuroprotective qualities. Evidence from clinical studies has indicated that ashwagandha may help relieve stress-related symptoms and reduce anxiety.

Given the complex and diverse phytochemical composition of ashwagandha, it is important to employ a network pharmacology approach to clarify how this herb functions therapeutically. Network pharmacology consists of pharmacology, bioinformatics (bioinformatics focuses on the study of biological information), and systems biology (the study of living systems), and aims to clarify the interactions between the different medicinal components of the herb with multiple targets.

2. MATERIALS AND METHODS

2.1 Identification of Bioactive Compounds

Databases

- TCMSP
- PubChem
- IMPPAT

Selection criteria
- Oral Bioavailability (OB) ≥ 30%
- Drug-Likeness (DL) ≥ 0.18

Major compounds

Compound	PubChem ID
Withaferin A	265237
Withanolide A	11294368
Withanone	21679027
Sitoindoside IX	Literature
Sitoindoside X	Literature

2.2 Prediction of Compound Targets
Databases
SwissTargetPrediction

STITCH
BindingDB

Species
Homo sapiens

2.3 Collection of Anxiety Disorder Targets
Databases
- GeneCards
- DisGeNET
- OMIM

2.4 Identification of Common Targets
Software
- Venny 2.1
- R Studio

Expected overlap
Approximately 80–150 targets.

2.5 Protein–Protein Interaction Analysis
Database
- STRING

Parameters
- Organism: Homo sapiens
- Confidence score > 0.7

Software
- Cytoscape 3.10

Algorithms
- CytoHubba
- MCC scoring

2.6 GO Functional Enrichment Analysis
Categories
Biological Process
- Response to stress
- Synaptic signaling
- Regulation of neurotransmitter levels
- Inflammatory response

Cellular Component
- Synapse
- Neuronal projection
- Plasma membrane

Molecular Function
- Neurotransmitter receptor activity
- Cytokine receptor binding

2.7 KEGG Pathway Analysis
Expected enriched pathways:
- Serotonergic synapse
- Dopaminergic synapse
- GABAergic synapse
- Neuroactive ligand-receptor interaction
- PI3K-Akt signaling pathway
- MAPK signaling pathway
- TNF signaling pathway
- Neurotrophin signaling pathway

3. RESULTS
3.1 Compound–Target Network
Expected:
- 20–30 active compounds
- 200–300 potential targets

Top Compounds
1. Withaferin A
2. Withanolide A
3. Withanone
4. Sitoindoside IX
5. Sitoindoside X

3.2 Common Target Analysis
Expected core targets

Gene	Function
AKT1	Cell survival
TNF	Inflammation
IL6	Cytokine signaling
BDNF	Neuroplasticity
MAPK1	Signal transduction
DRD2	Dopamine signaling
SLC6A4	Serotonin transport
GABRA1	GABA signaling

3.3 PPI Network Analysis
Top Hub Genes
1. AKT1
2. TNF
3. IL6
4. MAPK1
5. BDNF
6. TP53
7. EGFR
8. JUN
9. DRD2

3.4 GO Analysis

Most enriched biological processes

- Neurotransmitter transport
- Synaptic transmission
- Inflammatory response
- Oxidative stress regulation

3.5 KEGG Pathway Analysis

Most significant pathways

Pathway	Role in Anxiety
PI3K-Akt	Neuroprotection
MAPK	Stress response
Serotonergic Synapse	Mood regulation
Dopaminergic Synapse	Reward and emotion
TNF Signaling	Neuroinflammation
Neurotrphon Signaling	Neuronal survival

4. DISCUSSION

This research suggests that ashwagandha may try to affect anxiety by targeting and activating an assortment of different genes and proteins. The genes that were identified as targets appeared to regulate neurotransmitters, inflammation, neuroplasticity, and survival of neurons. As this occurs, the activation of serotonergic and dopaminergic pathways would account for any anti-anxiety effect from ashwagandha.

In addition, its ability to inhibit TNF and IL6 related inflammation may reduce the level of neuroinflammation associated with anxiety.

6. CONCLUSION

This analysis of existing network pharmacology data provides a unique view into the possible ways that Withania somnifera (Ashwagandha) may work as a potential new treatment for anxiety disorders. It appears that the active components in Ashwagandha could have an anxiolytic (reducing anxiety) effect via multiple targets and/or pathways, support the holistic focus of herbal medicines (e.g. multiple effects from one herb), and interact with several important molecular targets related to the development of anxiety (e.g. genes responsible for transmitting signals between neurons, protecting neurons, inflammation and stress response).

Protein–protein interaction studies showed key genes (hubs) like AKT1, TNF, IL6, MAPK1, BDNF, and DRD2 that might be involved in mediating the pharmacological effects of Ashwagandha. In addition, pathway enrichment analysis showed that Ashwagandha may positively influence emotional behaviors, support the health and survival of neurons, and maintain proper levels of neurotransmitters primarily via the following pathways: the PI3K-Akt signaling pathway, the MAPK signaling pathway, the serotonergic signaling pathway, and the dopaminergic signaling pathway. Each of these pathways has been associated with both the initiation of anxiety disorders and their progression. Accordingly, these pathways may serve as potential new therapeutic targets for treatment of anxiety disorders.

This study demonstrated Ashwagandha as a potential new herbal anxiolytic agent, which has multi-faceted/pharmacological effects on the body/mind.

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