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Insilico analysis of Ashwagandha (Withania somnifera (L.) Dunal) for its Balya activity with special reference to Cachexia

Research Article

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Abstract

Cachexia is a serious but under recognised consequence of many chronic diseases. The positive role of *Ashwagandha* in debility has been evaluated in several studies. But the underlying molecular mechanism is unclear. In the present study, network pharmacology is used to understand the molecular basis of its action. Methods: The phytoconstituents of *Ashwagandha* were screened from IMPPAT database and literature. The effective compounds were screened by drug likeness score and pharmacokinetic characteristics (ADMET). The target genes of effective compounds were predicted from BindingDB. The cachexia genes were found in gene-cards database, and cachexia related target genes were screened by comparison. Then the related pathways and correlation analysis were explored by the Genomes (KEGG) database. Finally, the networks of compound-target, target-pathway, and pathway-disease of *Ashwagandha* were constructed by Cytoscape software v3.7.2. Docking studies were carried out with PyRx software and analyzed in Biovia discovery studio visualizer. Results: The effective ingredients of *Ashwagandha* in cachexia were Withanolide S, Withanolide E, Withanolide D, Withasomniferol A and Beta sitosterol. The network analysis showed the highly modulating proteins were PTGS2, AR, PRKCB, JUN, TERT, NFE2L2, MDM2 and TNF which are related to cachexia and act on the pathways like MAPK signalling, MicroRNAs in cancer, cAMP signalling etc. The ligands and targets were retrieved from the PubChem, Protein Data Bank and docked using PyRx software. Conclusion: The present study is enabling to understand scientific evidence at the molecular level of *balya* action which has been proved clinically earlier.

Keywords: *Ashwagandha,* Cachexia, Network Pharmacology, Molecular docking.

Introduction

A complex wasting syndrome known as cachexia has been connected to a several long-term conditions, such as cancer, HIV, and chronic obstructive pulmonary disease (COPD)(1). Around 1% of the patient population, or about 9 million people, are afflicted with cachexia, which can be caused by any condition Globally(2). Cachexia significantly raises mortality and morbidity rates, lowers quality of life and increases healthcare costs.

Reduction in weight, a low BMI, weariness, and biochemical signs of systemic inflammation causes cachexia, which is characterised by an imbalance between metabolic demands and energy absorption. One important concept is that while malnutrition can be reversed with enough food, cachexia cannot. However, involuntary weight loss in people with numerous long-term conditions is frequent and severe enough to constitute a public health concern, regardless of how it is characterised (3). Treatments are urgently needed to prevent or at least slow the loss of muscle mass, increase strength, increase the ability for independent functioning, and extend survival rates in individuals with cachexia or vulnerable for acquiring it. Trials of potentially useful drugs often involve a small number of patients, and the majority of these studies are limited to individuals with cancer-related cachexia(4). Thus, there is a focus on traditional or new herbal supplements as well as proteins to help with the growth of muscle strength.

According to *Ayurveda*, Cachexia can be co-related to *Mamsa dhatu kshaya*. *Mamsa Dhatu* is entirely responsible for the body's strength, endurance, and immunity. The extent of *Bala* can be determined by lifting weights or engaging in physical activity, both of which require the presence of muscle and tendons, or *Mamsa dhatu*. *Acharya* also mentions characteristics of people with proper *Bala* and constitution as *Samamamsa*(5), which refers to the presence of proper and proportionate *Mamsa dhatu* in the body.

As all of the power, endurance, and *Bala* depend on *Mamsa dhatu*, it serves a very important role in providing immunity(6).

One of the most important therapeutic plants in *Ayurveda's* materia medica is *Withania somnifera* (L.)

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Dunal. It has been proven to be efficient and safe for a wide range of medical conditions from its ancient use to its present viewpoint, and is the best *Rasayana Dravya*(7,8). The herb *Withania somnifera* is known as *Balaprada* and *balya* in classical *Ayurveda* (which boosts immunity and strength)(9). *Acharya Charaka* included *Withania somnifera* under *balya* and *brimhaneeya* group in 4th chapter of *Sutra sthana*(10). Roots of the *Withania somnifera* plant demonstrate an increase in total muscle mass and strength, which helps in high resistance during physical exercise(11).

Herbal medicines have been utilised as a model for the drug development process for a very long time. In order to find new functional leads for cachexia, combinatorial sciences, high-throughput screening techniques, and the historical understanding from traditional medical systems are anticipated to facilitate the utilisation of herbal products and formulations in the drug development process.

Materials and Methods

Identification of *Withania somnifera* (L.) Dunal root bioactives

Bioactives present in *Withania somnifera* was screened through literature and database like IMPPAT(12) and downloaded in Structure Data File (SDF) format from the database PubChem(13) along with their phytochemistry. These phytochemicals were further investigated to determine their pharmacokinetic features.

Druglikeness of bioactives of *Withania somnifera*

Each phytoconstituent was screened for its Drug likeness property based on 'Rule of 5' using SWISS-ADME(14) and Molsoft software (15).

Target identification of bioactives from *Withania somnifera*

From BindingDB, the targets of all the phytoconstituents were found(16) with a probability of 0.7. UNIPROT was used to standardise the names of the predicted target genes. (17)

Disease targets were screened from Genecards(The Human Gene Database)(18). Upon querying the keyword cachexia, 7984 genes were retrieved and screened following Giftsscore, and the target with a Relevance score higher than the median was chosen as the target of cachexia since a target's relationship to a disease is more closely correlated with a target's relevance score in the Gene Cards database.

Estimation of overlapping genes

Genes that occur in both disease genes and drug targets are called overlapping genes. Overlapping genes were estimated from VENNY 2.1 mapping platform(19) by inserting screened disease targets in list 1 and drug targets in list 2, finally generating a venn diagram for each drug candidate.

Protein-protein interaction

PPI was constructed by introducing overlapping genes into the search tool of STRING (20) for the retrieval of interacting genes/proteins. The "cytoHubba" plug-in was used to calculate the degree value 10 from the PPI using Cytoscape 3.7.2 (21) and to find the top 10 molecular targets with a high level score, which were then chosen as hub-genes for cachexia.

KEGG Pathway analysis

The overlapping genes identified was queried in the STRING for KEGG (22) Pathway enrichment analysis, based on the literature, pathways were screened.

Network construction and analysis

The network between phytoconstituents, targets, and pathways was built using Cytoscape 3.7.2. In the process of building the network, any redundancy was eliminated. By establishing the network as directed and interpreting the entire network using edge count, the generated network was assessed using the "Network Analyzer" tool. Depending on the information used and the time taken to retrieve the data, the numbers of nodes for compounds, targets, and pathways may vary. Utilising several node types, the phytoconstituents, targets, and pathways were shown.

Docking Analysis

After analysing the network, for phytoconstituents having higher edge, information was obtained from the PubChem database and .sdf 3D formats are converted into .pdb using BIOVIA Discovery Studio visualizer (BDS)(23). Later highly enriched protein targets were queried on the RCSB protein databank(24). The proteins were prepared using BDS visualizer and Docking was carried using Pyrx software(25) to obtain compound-Target interaction and visualize for pose scoring minimum binding energy in BDS.

Results

Screening of bioactives and Druglikeness of *Withania somnifera* (L.) Dunal root

52 active components of *Withania somnifera* (L.) Dunal root were found after reading the literature study and were downloaded in sdf format. All 52 active components were analyzed in Molsoft software for the druglikeness properties, among them 16 compounds showed positive drug likeness properties along with positive Lipinski's rules and were subjected for further evaluation.

Target identification of bioactives from *Withania somnifera*

Protein targets for 16 phytocompounds were predicted using Binding DB server, linked to 89 disease targets. Screened phytoconstituents are summarized below in **Table 1.**

International Journal of Ayurvedic Medicine, Vol 14 (4), 2023; 988-993

Table 1: **Selected Phytoconstituents of *Ashwagandha* with their disease targets**

Phytoconstituents	Molecular Formula	Pubchem IDs	Name of targets
Withanolide D	C28 H38 O6	161671	AR, NFE2L2, PGR, PTGS2, PRKCB, TERT, JUN, TRPV4
Withaferine A	C28 H38 O6	265237	NFE2L2, PTGS2, PRKCB, TERT, JUN, TRPV4
Withanolide E	C28 H38 O7	301751	AR, MDM2, NFE2L2, PTGS2, PRKCB, TERT, JUN, TRPV4
Withanolide G	C28 H38 O5	21679023	AR, PTGS2, PRKCB
Withanolide P	C28 H38 O5	21679034	AR, MDM2, PTGS2, TNF
Withanone	C28 H38 O6	21679027	AR, NFE2L2, PTGS2, PRKCB, TERT, JUN, TRPV4
Withanolide S	C28 H40 O8	11049407	AR, MDM2, NFE2L2, PGR, PTGS2, PRKCB, TERT, JUN, TRPV4, TNF, VDR
Withanolide C	C28H36O5	101559583	AR
Withanolide L	C28 H36 O5	179575	AR, PTGS2, PRKCB, TRPV4
Galactitol	C6 H14 O6	11850	SRC
Withanolide F	C28 H38 O6	44562999	AR, PTGS2, PRKCB, TERT, JUN, TRPV4
Beta -Sitosterol	C29 H50 O	222284	AR, GRIN1, GRIN2A, MGLL, F2, SHBG, VDR
Somniferine	C15 H15 N O3	14106343	ACHE, ABCB1, CNR1
Withanolide A	C28 H38 O6	11294368	AR, NFE2L2, PTGS2, PRKCB, TERT, TRPV4
Withasomniferol A	C28 H38 O7	101710595	AR, NFE2L2, PTGS2, PRKCB, TERT, JUN, TRPV4
Withasomniferol C	C28 H38 O6	101710597	AR, PGR, PTGS2, PRKCB, TERT, TGFBR1, TRPV4

Estimation of overlapping genes

Twenty-one overlapping/common genes were identified by VENNY 2.1 for *Withania somnifera.*

Protein protein interaction

To determine the top 10 genes contributing to cachexia, *Withania somnifera* (L.) Dunal overlapping targets and its metabolites were examined using the STRING database to create a PPI network with a moderate confidence score (0.4). PPI was then visualised using Cytoscape to create a network for Withania somnifera that had 21 nodes and 62 edges. The top 10 hub genes with the highest "degree" score were found using the Cytohubba plugin are; TNF, SRC, PTGS2, JUN, AR, CNR1, PGR, MDM2, PRKCB, GRIN1.

KEGG Pathway analysis

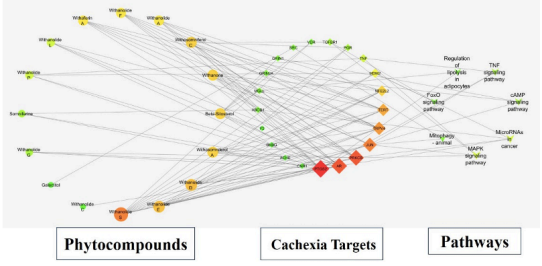
On 21 drug and disease intersecting targets, KEGG pathway enrichment analysis was done, and 98 enrichment pathways were obtained, in which 7 pathways were associated with Cachexia. 7 pathways are MicroRNAs in cancer, MAPK signalling, TNF signalling, cAMP signalling, Mitophagy – animal, FoxO signalling , Regulation of lipolysis in adipocytes. Highly enriched are MicroRNAs in cancer, MAPK signalling pathway modulating PTGS2, PRKCB, AR, JUN, TERT, NFE2L2, MDM2 and TNF Targets.

Seven pathways implicated in cachexia were discovered using gene set enrichment analysis **Table 2.**

Table 2: Gene-set enrichment analysis of Cachexia Disease Pathway

	Pathways	No of targets	Targets
hsa05206	MicroRNAs in cancer	4	MDM2, PRKCB, PTGS2, ABCB1
hsa04010	MAPK signalling pathway	4	PRKCB, JUN, TGFBR1, TNF
hsa04668	TNF signalling pathway	3	PTGS2, JUN, TNF
hsa04024	cAMP signalling pathway	3	JUN, GRIN1, GRIN2A
hsa04137	Mitophagy - animal	2	JUN, SRC
hsa04068	FoxO signalling pathway	2	MDM2, TGFBR1
hsa04923	Regulation of lipolysis in adipocytes	2	MGLL, PTGS2

Figure 1: Network interaction between phytocompounds from *Ashwagandha* with their modulated protein targets and pathways



Construction of Network and analysis

7 distinct pathways were discovered by network enrichment analysis and were modulated by targets related to cachexia. The constructed network contains 109 edges. Among them, 89 are compound-protein interactions and 20 protein pathway interactions. The constructed network included 44 nodes, 109 edges representing 7 pathways and 21 targets. Withanolide S had the highest edge count, interactions were found with 11 targets represented in **Figure 1.**

Docking analysis results

Molecular docking performed on phyto-compounds with therapeutic targets i.e., PTGS2, AR, JUN, TERT. Among the screened molecules Withanolide-D scored the lowest BE of -11 kcal/mol with TERT via forming interaction with ALA223, ALA226, ILE46, ILE218 and TYR183 (**Figure 2)**. Withanolide D also scored the lowest BE of -9.1 kcal/mol with PTGS2 via forming interaction with LYS97 and HIS356 **(Figure 3)**. Similarly, Withanolide E scored the lowest BE of -7.2kcal/mol with AR via forming interaction with TYR90, PRO45, PHE102, GLY103 **(Figure 4)**. Similarly, Withasomniferol A scored the lowest BE of -8.7 kcal/mol with JUN via forming interaction with LYS308, CYS245, VAL303, SER299, PHE271 and SER270 **(Figure 5)**. The binding affinity of the Phytochemicals and the Targets which were highly modulated are tabulated (Table 3)

Figure 2: 2D and 3D representation of Interaction of TERT receptor with Withanolide D

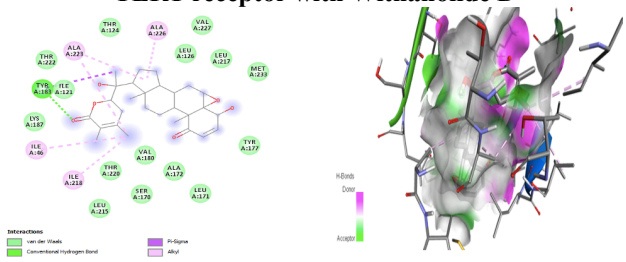


Figure 3: 2D and 3D representation of Interaction of PTGS2 receptor with Withanolide D

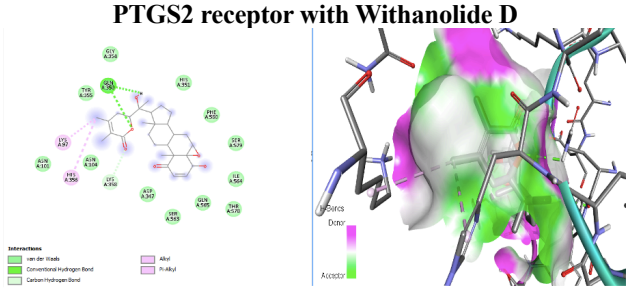


Figure 4: 2D and 3D representation of Interaction of AR receptor with Withanolide E

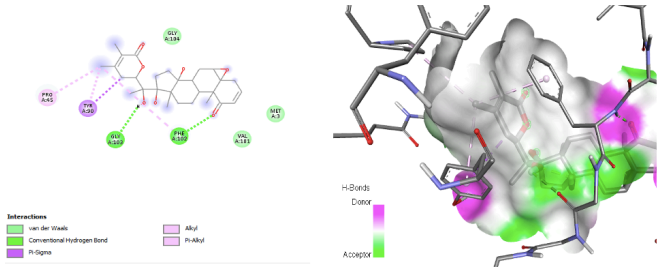


Figure 5: 2D and 3D representation of Interaction of JUN receptor with Withasomniferol A

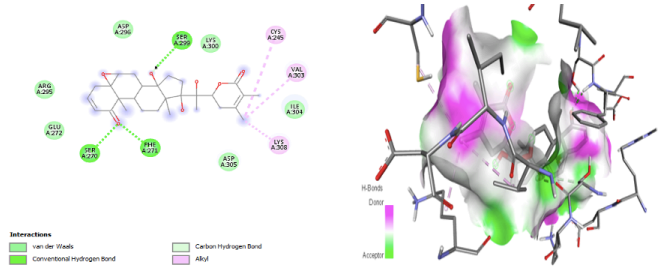


Table 3: Binding affinity of the highly modulated Phytocompounds and the Targets

Target	Phytocompounds	Binding Energy
PTGS2	Withanolide D	-9.1
	Withanolide S	-8.7
	Beta sitosterol	-8.3
PRKCB	Beta sitosterol	-8.7
AR	Withasomniferol A	-8.3
	Withanolide E	-7.2
	Beta sitosterol	-6.7
JUN	Withasomniferol A	-8.7
	Withanolide E	-8
	Withasomniferol C	-7.7
	Withaferine A	-7.4
TERT	Withanolide D	-11
	Withaferine A	-9.2

Discussion

Advances in treatment are urgently needed for people around the world due to cachexia's high prevalence and extremely high mortality(26). The main causes of cachexia's onset involve a rise in inflammation, a reduction in muscle protein synthesis, and problems with glucose, protein, and lipid metabolism. Skeletal muscle atrophy is brought on by the overactivation of several intracellular proteolytic processes, including the ubiquitin-proteasome and autophagy. As a defence mechanism, anabolic capacity, autophagy, and myogenesis are activated(27). However, it is being thought about as a possible treatment for cachexia to use nutritional supplements or medications that can control catabolic processes, cell damage, and inflammation. The goal of *Ayurveda's* holistic yet personalised approach is to improve immunity while restoring and maintaining the homeodynamics of the body.

Withania somnifera is a *rasayana* and regenerating herb in Ayurveda, and the part used is the root. *Rasayana* strategy in this regard is used in the practice of *Ayurveda* as both preventive and therapeutic purpose(28). *Withania somnifera* contains numerous pharmacologically useful phytochemicals, including alkaloids like isopelletierine and anaferine, saponins, steroidal lactones (withanolides, withaferins). *Withania* has the ability to withstand a variety of muscle stressors due to its antioxidant, anti-inflammatory, immunomodulatory, and adaptogenic qualities, which are assumed to be a result of the withanolides (29).

Numerous studies have proven the clinical efficacy of ashwagandha extracts in regulating body fat and muscle. A study on healthy volunteers found that they had stronger muscles and less fat, as well as lower levels of total and LDL cholesterol(30).

The effects of *Boswellia serrata, Cissus quadrangularis* and *Withania somnifera* as a herbal combination on Sarcopenia have been shown to significantly increase muscle mass, grip strength, motor coordination, gait, locomotor activity, and endurance, indicating the herbal combination's potential to treat the pathophysiological changes connected to Sarcopenia(27).

According to several research, *Ashwagandha* may enhance strength and body composition(11). Another study revealed that those who frequently ingested *Ashwagandha* developed noticeably stronger muscles.

The effective phytoconstituents of *Ashwagandha* in cachexia were Withanolide S, Withanolide E ,Withanolide D, Withasomniferol A and Beta sitosterol. The network analysis showed the highly modulating proteins were PTGS2, PRKCB, AR, JUN, TRPV4, TERT, NFE2L2, MDM2 and TNF related to cachexia which acts on the pathways like MAPK signalling, MicroRNAs in cancer, cAMP signalling, TNF signalling, FoXo signalling and Mitophagy.

Increased levels of inflammatory cytokines including Interleukin (IL)-6, Tumour Necrosis cause (TNF), and IL-1 are regarded to be a major contributing cause to cachexia. One of the characteristics of cancer cachexia is muscle atrophy, which is caused by proteolysis carried out by the ubiquitin-proteasome pathway (UPP) via signalling molecules activated by cytokines such as NF-kB, p38MAPK or JAK-STAT3, and the autophagy-lysosome route (ALP)(31).

Inflammatory cytokines have a significant impact in loss of skeletal mass in chronic illnesses. Since muscle precursor cells play a role in muscle growth and regeneration. Therefore downstream signalling pathways may be utilized to promote muscle regeneration. in individuals with acute injury or muscular dystrophy. In individuals with acute injury or muscular dystrophy, cAMP also plays a role in muscle growth and regeneration, which is mediated by muscle precursor cells(32).

According to data, p38 MAPK represents a key therapeutic target for the muscle loss brought on by cancer(33). The likelihood of cachexia and muscle atrophy in cancer patients is hypothesised to be increased by a "non-coding RNA" called micro ribonucleic acid (miRNA). There are numerous inflammatory and illness pathways that have an impact on how miRNA regulates proteins(34). Recent studies have shown that cachectic skeletal muscle has elevated forkhead box O (FoxO) signalling. In cancer cachexia and sepsis, expression of DN FoxO decreased the mRNA levels of atrogin-1, MuRF1, cathepsin L, and/or Bnip3 and avoided muscle fibre atrophy. By blocking FoxO transcriptional activity, muscular hypertrophy is stimulated and muscle fibre loss during cachexia is avoided(35).

PTGS2 gene also known as COX2, is upregulated during inflammation and is the target of many non-steroidal anti-inflammatory drugs. COX2 inhibitors are type of NSAIDS used to treat inflammation(36). The PI3K/Akt-mTOR pathway is activated by the AR target genes insulin growth factor-1 (IGF-1) and insulin growth factor-1 receptor (IGF-1R), which drive muscle hypertrophy(37). Previous study show that the pretranslational level of JunD expression is down regulated in cachexia-related muscle wasting, and that oxidative changes to JunD may lead to JunD ubiquitination(38). The unique C/EBP regulation of TERT in skeletal muscle and the discovery of C/EBP as a prospective therapeutic target for the treatment of muscular disorders like rhabdomyosarcoma highlight the importance of this protein(39).

Conclusion

This article explained the mode of action of *Ashwagandha* on Cachexia through network pharmacological analysis and docking. The important key compounds of *Ashwagandha* were Withanolide D, Withanolide E and Withasomniferol A and the highly modulating were PTGS2, AR, JUN, TERT proteins. Ashwagandha can be given as adjunct in patients undergoing chemotherapy. Insilico analysis could be useful tool for understanding the mode of action of pharmacological activities and drugs described in traditional systems of Medicine like *Ayurveda,* thus paving a path for their scientific validation.

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References

1. Rohm M, Zeigerer A, Machado J, *et al*. Energy metabolism in cachexia. *EMBO Rep* 2019; 20:e47258.
2. Anker SD, von Haehling S. Efforts begin to sprout: publications in JCSM on cachexia, sarcopenia and muscle wasting receive attention. J Cachexia Sarcopenia Muscle. 2014; 5:171–6.
3. Farkas J, von Haeling S, Kalantar-Zadeh K, Morley JE, Anker SD, Lainscak M. Cachexia as a major public health problem: frequent, costly, and deadly. J Cachexia Sarcopenia Muscle.2013;4:173–8.
4. Von Haehling S, Anker SD. Treatment of cachexia: an overview of recent developments. Int J Cardiol. 2014;in press.
5. Tripathi Bramhanand, Charak Samhita with Hindi Commentary, Sutra Sthana 21, Choukhamba Surbharti Prakashan Varanasi, reprint 2004.
6. Mohit Kumar, Sanjay Kumar Agri. Review Article on Mamsa Dhatu -Ayurveda and Modern view. J Ayurveda Integr Med Sci 2019; 1:73-77.
7. Krishnapriya S, Senthil K. Therapeutic potential of *Withania somnifera* (Linn) Dunal (Ashwagandha) in historical perspective and pharmacological evidence.10. *Ann. Ayurvedic Med.*; 2021.135p.
8. Jyotsna et al. A review article on Rasayana therapy in ayurveda. 5(5). WJPMR; 2019.39-42p.
9. Girdhari Lal Gupta, Rana A C. *Withania somnifera* (Ashwagandha): A Review. 1(1). *Pharmacogn Rev*; 2007. 129-136p.
10. Yadavji Trikamji. Charaka Samhitha by Agnivesha.4th edition, Varanasi; Chaukambha Sanskrit Sansthan publishers, 1994.
11. Wankhede S, Langade D, Joshi K, Sinha S R, Bhattacharyya S. Examining the effect of *Withania somnifera* supplementation on muscle strength and recovery: a randomized controlled trial, Sports, *Nutr. Rev. J.,* 2015;12:43.
12. Karthikeyan Mohanraj, Bagavathy Shanmugam Karthikeyan, Vivek-Ananth R.P., Bharath Chand R.P.,

Aparna S.R., Mangalapandi P. and Areejit Samal, IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry and Therapeutics, Scientific Reports (2018), 8:4329

13. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. PubChem 2023 update. Nucleic Acids Res., (2023). 51(D1), D1373–D1380.
14. Daina, Antoine., Michielin, Olivier., Zoete, Vincent. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports. (2017). 7. 42717. 10.1038/srep42717.
15. ICM-Pro, Molsoft, L.L.C. http://www.molsoft.com/ has been cited by the following article: TITLE: Estimated Binding Energies of Drug-Like and Nondrug-Like Molecules in the Active Site of HIV-1 Integrase, 1BIS.pdb, and Two Mutant Models: Y143R and N155H.
16. Gilson MK, Liu T, Baitaluk M, Nicola G, Hwang L, Chong J. BindingDB in 2015: A public database for medicinal chemistry, computational chemistry and systems pharmacology. Nucleic Acids Res 2015;44(D1):D1045-53.
17. Boutet E, Lieberherr D, Tognolli M, Schneider M, Bansal P, Bridge AJ, et al. UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: How to use the entry view. Methods Mol Biol 2016;1374:23-54.
18. Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, Nativ N, et al. 2010. GeneCards Version 3: the humangene integrator. Database. 2010:baq020.
19. Oliveros, J.C. (2007-2015) Venny. An Interactive Tool for Comparing Lists with Venn's Diagrams.
20. Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017; 45(D1):D362–D368.
21. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research. 2003;13(11):2498–504.
22. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res.2017;45(D1):D353–61.
23. Systèmes D. Biovia, discovery studio modeling environment. Dassault Systèmes Biovia: San Diego, CA, USA; 2016.
24. Berman H.M., Westbrook J., Feng Z., Gilliland G., Bhat T.N., Weissig H., Shindyalov I.N., Bourne P.E. The Protein Data Bank Nucleic Acids Research, (2000), 28: 235-242. (rcsb.org)
25. Dallakyan.S, Olson A. J, "Small-molecule library screening by docking with PyRx," Methods in Molecular Biology, 2015. vol. 1263, pp. 243–250,
26. Von Haehling S, Anker SD. Prevalence, incidence and clinical impact of cachexia: facts and numbers-update 2014. J Cachexia Sarcopenia Muscle. 2014 Dec;5(4):261-3.
27. Azeemuddin MM, Rao CM, Rafiq M, Onkaramurthy M, Singh P, Baig MR, Babu UV. A herbal combination attenuates muscle atrophy and cancer cachexia: A preclinical study. J Appl Pharm Sci, 2022; 12(04):119–126.
28. Saggam A, Tillu G, Dixit S, Chavan-Gautam P, Borse S, Joshi K, et al. *Withania somnifera* (L.) Dunal: a potential therapeutic adjuvant in cancer.J Ethnopharmacol 2020;255:112759.
29. Panda V, Deshmukh A, Hare A, Singh S, Hingorani L, Sudhamani S. Effect of *Withania somnifera* hydroalcoholic extract and other dietary interventions in improving muscle strength in aging rats. J Ayurveda Integr Med. 2021 Oct-Dec;12(4):623-632.
30. Wang J, Zhang H, Kaul A, Li K, Priyandoko D, Kaul SC, Wadhwa R. Effect of Ashwagandha Withanolides on Muscle Cell Differentiation. Biomolecules. 2021 Oct 4;11(10):1454.
31. Advani SM, Advani PG, VonVille HM, Jafri SH. Pharmacological management of cachexia in adult cancer patients: a systematic review of clinical trials. BMC Cancer. 2018 Nov 27;18(1):1174.
32. Berdeaux R, Stewart R. cAMP signalling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. Am J Physiol Endocrinol Metab. 2012 Jul 1;303(1):E1-17.
33. Liu Z, Sin KWT, Ding H, Doan HA, Gao S, Miao H, Wei Y, Wang Y, Zhang G, Li YP. p38β MAPK mediates ULK1-dependent induction of autophagy in skeletal muscle of tumor-bearing mice. Cell Stress. 2018 Oct 10;2(11):311-324.
34. Sutandyo N. The role of microRNA in cancer cachexia and muscle wasting: A review article. Caspian J Intern Med. 2021 Mar;12(2):124-128.
35. Reed SA, Sandesara PB, Senf SM, Judge AR. Inhibition of FoxO transcriptional activity prevents muscle fiber atrophy during cachexia and induces hypertrophy. FASEB J. 2012 Mar;26(3):987-1000.
36. Li R, Xie J, Xu W, Zhang L, Lin H, Huang W. LPS-induced PTGS2 manipulates the inflammatory response through trophoblast invasion in preeclampsia via NF-κB pathway. Reprod Biol. 2022 Dec;22(4):100696.
37. Yin L, Lu L, Lin X, Wang X. Crucial role of androgen receptor in resistance and endurance trainings-induced muscle hypertrophy through IGF-1/IGF-1R- PI3K/ Akt- mTOR pathway. Nutr Metab (Lond). 2020 Mar 30;17:26.
38. Ramamoorthy S, Donohue M, Buck M. Decreased Jun-D and myogenin expression in muscle wasting of human cachexia. Am J Physiol Endocrinol Metab. 2009 Aug;297(2):E392-401.
39. Slivitzky, Kira. The Regulation of Telomerase Reverse Transcriptase (TERT) by CCAAT/Enhancer Binding Protein B (C/EBPβ) During Skeletal Muscle Differentiation. Canada, Université d'Ottawa / University of Ottawa, 2017.

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