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Original Article

In vitro and *in silico* Approach to Determine the Molecular Mechanism of Action of Yashtimadhu Moola Churna (Root Powder of *Glycyrrhiza glabra* L.)

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ABSTRACT

Background: Wearing spectacles due to refractive error has been a common thing now a days. Not only among geriatrics but also among paediatrics wearing spectacles due to refractive error has been increasing due to indulgence in strenuous activities in front of computer, mobile phones, television and video games. **Aim:** With the increasing in this trend only spectacles wouldn't solve the problem, we need medicines that improve the eyesight of common people. *Yastimadhu* (*Glycyrrhiza glabra* L.) is one of the Drug that is mentioned in classical treatise that improves eyesight (*chakshushya*). Though it is indicated in eye disorder, the mechanism behind the plant is still unclear. Thus, so as to establish the property pharmacologically, *in vitro* and *in silico* analysis was performed. **Materials and Methods:** *Yastimadhu* (*Glycyrrhiza glabra*) was procured from GMP certified pharmacy and followed by that ash value, Alcohol and Water-soluble extract, anti-oxidant activity and followed by plant related and diseases related targets were procured from databases and uploaded into the database to obtain overlapping targets of the two refractive errors i.e. astigmatism and myopia. With the common targets the further protein network, hub gene prediction was done. Followed by that KEGG pathway prediction the topological analysis was performed; degree layout was considered and molecular docking was done. **Results and Discussion:** The basic physicochemical analysis results were within the limits of API, while the anti-oxidant activity gave IC_{50} value of 137.86 µg/mL. A total of 467 plant targets and 808 targets related to astigmatism, 1451 targets related to myopia were uploaded into database and 26 overlapping targets related to astigmatism and 28 related to myopia was obtained. Followed by this when upon predicting top 10 hub genes yielded ESR1, HIF1A, TNF, CTNNB1 and IL6 as major targets in both the disease. While performing KEGG pathway analysis, pathways in cancer was common among both the disease. After topological analysis, once the docking was performed the binding affinity was calculated and it revealed that liquritin had the highest binding affinity of -9.3 kcal/mol towards ESR1. **Conclusion:** Though the study has given primitively that *Yastimadhu* has role on the disease and improves eyesight further clinical evaluation is required to establish the study.

Keywords: Computational Pharmacology, *Glycyrrhiza glabra* L., *In vitro* Analysis, Molecular Docking, *Yastimadhu*.

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INTRODUCTION

Refractive errors have been a common ailment all around the world, globally about 153 million people suffer with visual impairment and 410 million people have suffered with performing the day-to-day activity without spectacles according to a meta-analysis in the year 2010 (Cochrane *et al.*, 2010). Compared to the year 2010 there is relatively a great increase in the number cases till 6.5% in a meta-analysis in the year 2023. Especially the percentage

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of people wearing spectacles are quite higher in the countries with higher income (Bourne *et al.*, 2025). Refractive errors are majorly classified into Myopia, Hypermetropia, Astigmatism, Anisometropia and Presbyopia (Wedner *et al.*, 2003). In this Myopia i.e. short sightedness and Astigmatism i.e. parallel rays from cornea doesn't converge at a point in retina (Wajuihian, 2017). A survey in Germany within the age group of 35-74 years revealed that among the refractive errors Myopia was on higher side when compared to hypermetropia (Schiefer *et al.*, 2016). The main management of these disorders is either the patient should opt for surgery or else the patient should purchase his/her spectacles (Cochrane *et al.*, 2010). There is a chance that this may lead to increase in the refractive error on a long time, due to the lack in drugs that help in promoting the eye sight. In Ayurveda, the single drug therapy especially in the form of *rasayana* that are to be taken during the age of 51 - 60 years, and the suggested drug to be taken during this time is *Triphala* and *shatavari* (Mishra *et al.*, 2016). This *rasayana* is prescribed in this particular age so as to improve the eyesight, but this was during ancient era. Currently, due to our irregular lifestyle and day-to-day routine the number of cases has been increasing and we need newer drugs that promote eyesight and comparatively the availability of the plant also matters due to the surge in substitute drugs.

Yastimadhu moola (root of *Glycyrrhiza glabra* L.) is one such plant that is indicated in *Ayurveda* as *Chakshushya* (promotes eyesight and strength) according to *Bhavapraksha nighatu* (Murthy, 2012). In Astanga Hridaya, it is mentioned in external applications such as *Anjana* (collyrium) and *Bidalaka* (application of paste on eyelids except eyelashes) (Murthy, 2012). Though it is mentioned in both single drug and compound formulations for improving eyesight, the mechanism behind the drug is still unclear. Thus, so as to establish the mechanism behind the plant's role on how it works in improving eye sight, basic physico-chemical, anti-oxidant activity and followed by that *in silico* activity on 2 refractive errors i.e. myopia and astigmatism was performed.

MATERIALS AND METHODS

Here, first the raw materials were purchased followed by that *in vitro* and *in silico* examination of the *Yastimadhu moola churna* was done and the layout of the process is illustrated in Figure 1.

Ethical statement

Since the preparation, *in vitro* and *in silico* investigation doesn't involve any human or animal participants the permission from Institutional Ethical Committee and Institutional Animal Ethical Committee was not required.

Procuring *yastimadhu,* Physico-chemical and Anti-oxidant analysis

The drug *yastimadhu* was procured from KLE Ayurveda GMP certified pharmacy and followed by that physico-chemical and anti-oxidant activity by DPPH method was performed as per API volume 3 (Duke *et al.*, 1992).

Statistical analysis

The anti-oxidant values were tabulated from 500 µg/mL to 3.09625 µg/mL and the IC_{50} values were calculated. Calculations were made as per the standard formula, the IC_{50} was calculated using the formula:

$$IC_{50}=C1 + (50-I1/I2-I1) \times (C2-C1)$$

Procuring targets related to plants

The databases IMPPAT 2.0 and Dr. Dukes was used to acquire the phytochemicals related to plant (Mohanraj *et al.*, 2018; Kim *et al.*, 2025). Later, these phytochemicals related SMILES (Simplified Molecular Input Line Entry System) were procured from PubChem (Fu *et al.*, 2024). These SMILES were then loaded into Swiss target prediction for acquiring targets related to the particular phytochemicals after filtering the phytochemicals on the basis of QED (Qualitative Estimation of Drug likeliness), Lipinksi rule of 5, HIA (Human Intestinal Absorption), Blood brain barrier and Eye sensitivity from ADMET Lab 3.0 (Gfeller *et al.*, 2014). Furthermore, these phytochemical related SMILES were uploaded into Swiss target prediction and retrieved targets related to these phytochemicals, and additionally when the duplicate targets were removed a total of targets were found at last (Stelzer *et al.*, 2016).

Acquiring disease target and overlapping target

The disease related targets were acquired from the databases HPA (Human Protein Atlas) and Gene cards and they were filtered on the basis of cut-off score and the duplicates were removed followed by this the targets related to the disease and the target related to plants were uploaded into Venny 2.1.0 for acquiring overlapping targets (Thul *et al.*, 2017; Oliveros, 2007; Szklarczyk *et al.*, 2023).

Hub genes and Protein interaction

The common targets were then uploaded into STRING database with selecting multiple proteins and species as *"Homo sapiens" "Homo sapiens"* the interaction between the common targets were predicted and the interaction is viewed (Shannon *et al.*, 2003). Then the network is sent to Cytoscape 3.10.4 for final visualisation of the Protein interaction (Chin *et al.*, 2014). Then Cytohubba was used to predict the top 10 hub genes of the Protein interaction (Sherman *et al.*, 2022). The rank of the top 10 hub genes are noted for further network construction.

KEGG Pathway prediction

The common targets were then uploaded into DAVID Bioinformatics and then upon selecting the species and the gene list as Official gene symbol the pathways related to the common

targets were obtained (Tang *et al.*, 2023). The *p* value, count and the pathways action on the disease were noted for further topological analysis.

Topological analysis

Upon acquiring the overlapping targets, Phytochemicals and Pathways the topological analysis of Target-Pathway-Compound network was Constructed with Cytoscape 3.10.4. Based on the degree layout the compounds were further filtered for docking, followed by that the degree betweenness and the edge count was also noted for the compound and the targets. The chemicals with highest degree layout were taken for further molecular docking process.

Molecular docking

The targets from top 10 hub genes and the chemicals that had the highest degree layout were made to dock. By downloading the 3D structures of the ligand and the targets from PubChem and RCS PDB respectively, the targets were taken further into Autodock tool 1.5.7 for adding the hydrogen bond and kollmann charges and removing the hetero atoms and water molecules (Burley *et al.*, 2023; Dassault systems, 2021). Later the targets that are uploaded into PyRx software where it is made to dock with the 3d structures of the phytochemicals (Morris *et al.*, 2009). Later the docking is visualised with the Discovery Biovia studio for 2D and 3D structures and the binding affinity of each target towards ligand is tabulated and the highest binding affinity between the ligand - target is also noted (Dallakyan *et al.*, 2015).

RESULTS

Physico-chemical analysis and Anti-oxidant activity

The sample i.e. *yastimadhu moola chruna* was assessed for various standards including ash value, total moisture content, acid insoluble ash, water soluble extract and alcohol soluble extract and all the tests were under the API norms. Followed by that with the prepared alcohol soluble extract the anti-oxidant activity was performed and the *yastimadhu* had IC_{50} of 137.86 µg/mL. The physico-chemical and the anti-oxidant activity of the *Yastimadhu moola churna* are tabulated in Table 1.

Plant targets

The databases IMPPAT and Dr. Dukes had 504 targets on the whole, subsequently removing the metals, water molecules and fat molecules from the list. Both the chemicals were merged and the duplicates were removed, finally 441 chemicals were obtained. After this the 257 SMILES related to phytochemicals were retrieved from PubChem, while the remaining 184 chemicals didn't had related SMILES. Later these 257 SMILES were uploaded into ADMET Lab 3.0 for assessment of Absorption, Distribution, Metabolism, Excretion and Toxicity and filter them according to the parameters such as Lipinski rule of 5, QED with cutoff score 0.67 ≥, Human Intestinal Absorption, F30, Eye sensitivity and Blood brain barrier with a medium cutoff score of 0.50 ≥, a total of 21 phytochemicals were found. Furthermore, these 21 phytochemical related SMILES were uploaded into Swiss target prediction and retrieved 1292 targets related to these phytochemicals, and additionally when the duplicate targets were removed a total of 467 targets were found at last.

Acquiring disease targets and overlapping targets

Among the refractive errors Myopia and Astigmatism were taken for acquiring disease related targets, these two diseases were searched into Gene cards and Human Protein Atlas databases. With a cutoff score of 2, a total of 1501 targets related to myopia and 808 targets related to Astigmatism was obtained from the databases after combining and removing the duplicates. The plant related targets and the disease related targets were uploaded into Venny 2.1.0 for acquiring overlapping common targets, on a total 58 overlapping targets was found in between Myopia and plant while 26 overlapping targets was found between plant and Astigmatism. The overlapping targets between the diseases is given in the Figure 2.

Hub genes and Protein interaction

The protein interaction between the targets was visualised by uploading the targets into STRING database followed by that the PPI network was further visualised in Cytoscape 3.10.4. Upon visualisation the target CTNNB1 had the most interaction among the 58 targets in Myopia while IL6 had the most interaction in Astigmatism. Subsequently, by using Cytohubba for prediction of top 10 hub genes gave further more precision of the main targets which are involved in the disease such as IL6, HIF1A, ESR1, MTOR, CTNNB1, TNF, SRC, MMP9, IL1B and KDR for myopia while astigmatism had targets such as CTNNB1, ESR1, MTOR, TNF, CDK4, CDK6, PIK3CA, BRCA1 and TERT. Among this CTNNB1 had a score of 88094 and it had the top most score, while in myopia hub genes had ESR1 the highest score of 22537153562 but the most interaction among the PPI network i.e. IL6 target

Table 1: Physico-chemical and Anti-oxidant activity of *Yastimadhu moola churna* (*Glycyrrhiza glabra* root powder).

Test	Results
Ash value	7.796%
Moisture content	12.886%
Acid insoluble ash	1.112%
Water soluble extract	37.524%
Alcohol soluble extract	20.109%
Anti-oxidant activity	
Concentration (µg/mL)	Result
125	49.23
250	51.63

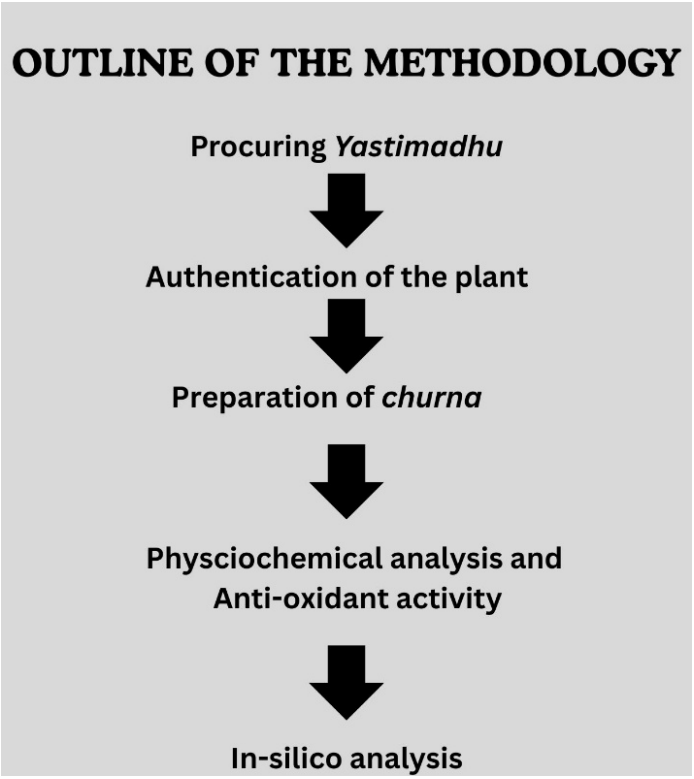


Figure 1: Outline of the methodology.

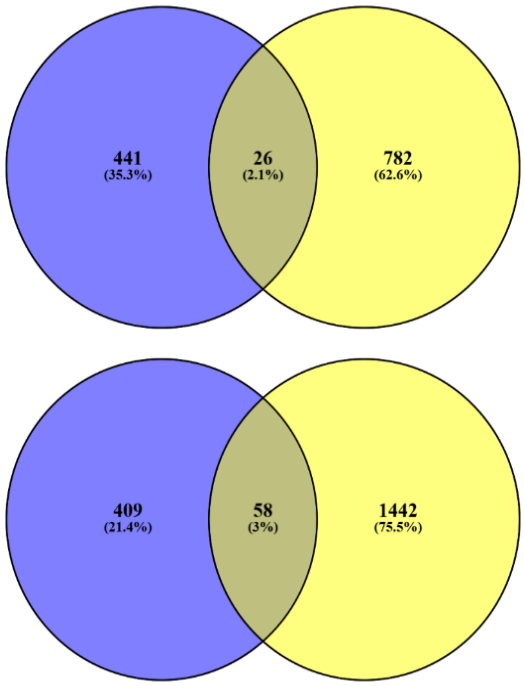


Figure 2: The above picture depicts the overlapping targets between plant and astigmatism, while the below one is the common targets between myopia.

was second among the hub gene ranking. The PPI and Hub genes are depicted in Figure 3.

KEGG pathway

When the overlapping targets were uploaded into DAVID Bioinformatics the KEGG pathways were acquired, in myopia there were a total of 135 pathways while in astigmatism there were a total of 67 pathways among these both disease, pathways in cancer and proteoglycans in cancer were the top KEGG pathways. Among these the top 10 pathways of both the disease were filtered and further they were taken for topological analysis. The pathways in cancer of myopia had 24 targets with 42.31% of genes while astigmatism KEGG pathways in cancer had 11 targets with 41.38% of genes.

Target - compound - pathway analysis

The total 26 targets, 15 compounds and 10 KEGG pathways of "astigmatism" 58 targets, 15 compounds and 10 KEGG pathways of "myopia" were uploaded separately into Cytoscape 3.10.4 and followed by that degree layout between the pathway, compound and target analysis was done. In myopia, 10 compounds had degree layout i.e. the interaction between targets was 101, while Beta-carotene and asparagine degree layout of 76 and 55 respectively. In astigmatism, 10 compounds had degree layout of 101 while glabric acid had the lowest degree among the both diseases with a degree layout of 1. The KEGG pathway analysis of "astigmatism" had 11 gene count with pathways of cancer, while 5 gene count with chronic myeloid leukaemia, while in "myopia" 11 gene count was the lowest interaction with HIF signalling pathway. The TPC analysis of astigmatism and myopia is depicted in Figure 4.

Molecular docking

After literature review and based on the degree layout on the topological analysis, 4 phytochemicals of *Glycyrrhiza glabra*, Liquiritin, 18-β-Glycyrrhetinic Acid, Isoglycyrol and Tetramethyl-pyrazine was considered as ligands and targets for astigmatism i.e. TNF, ESR1 and CTNNB1 were chosen based on the hub gene prediction while targets such as ESR1, HIF1A and IL6 were chosen for myopia. The phytochemicals 3D structures were downloaded from PubChem, followed by that targets were downloaded from RCS PDB with a PDB of 1JDH - CTNNB1, 3DT3 - ESR1, 1QLB - HIF1A, 1ALU - IL6 and 2TNF - TNF. With Autodock tool 1.5.7 the water molecules were removed, polar atoms were added Kollmann charges were added. PyRx software was further used to make the interaction between ligand and target, the binding affinity was noted. ESR1 had the highest binding affinity -9.3 kcal/mol towards liquiritin ligand, while the lowest was for Tetra methyl pyrazine with a binding affinity of -4.3 kcal/mol towards HIF1A target. The binding affinity of ligand and target interaction of "Astigmatism" and "Myopia" is given in the Tables 2 and 3 respectively. The visualization of ESR1 towards Liquiritin and HIF1A towards Isoglycyrol is depicted in Figure 5.

DISCUSSION

Yastimadhu (*Glycyrrhiza glabra* L.) is a plant that is mentioned in all *Bruhatrayees* and *Laghutrayees* (major classical treatise of Ayurveda), especially in Charaka Samhita out of 50 *Mahakashayas* mentioned it is there in 13 *mahakashayas*, one among the *mahakashyas* is *jeevaniya mahakashaya* (*Kashayas* that promote longevity), being said the drug may also promote the longevity of eyes (Sharma and Dash, 2014). And moreover, it is mentioned as *Chakshushya* (helps in improving eyesight) as per *Bhavapraksha*. *Yastimadhu* (*Glycyrrhiza glabra*) is *Madhura rasa* (sweet taste), *Madhura vipaka* (sweet in post digestion effect), *sheeta virya* (cold in potency) and *Guru, Snigdha guna* (heaviness and unctuousness in property) and it helps in pacifying *vata* and *pitta* (Srikantha Murthy, 2012). Because of the *pittahara* property it may help in pacifying the aggravated *alochaka pitta* (the *dosha* that is located in eyes) which may be one of the cause of reduction in eyesight in refractive error. Furthermore, to add *Madhura rasa, Guru* and *Snigdha guna* and *sheeta virya* will further more helps in reducing the *pitta*.

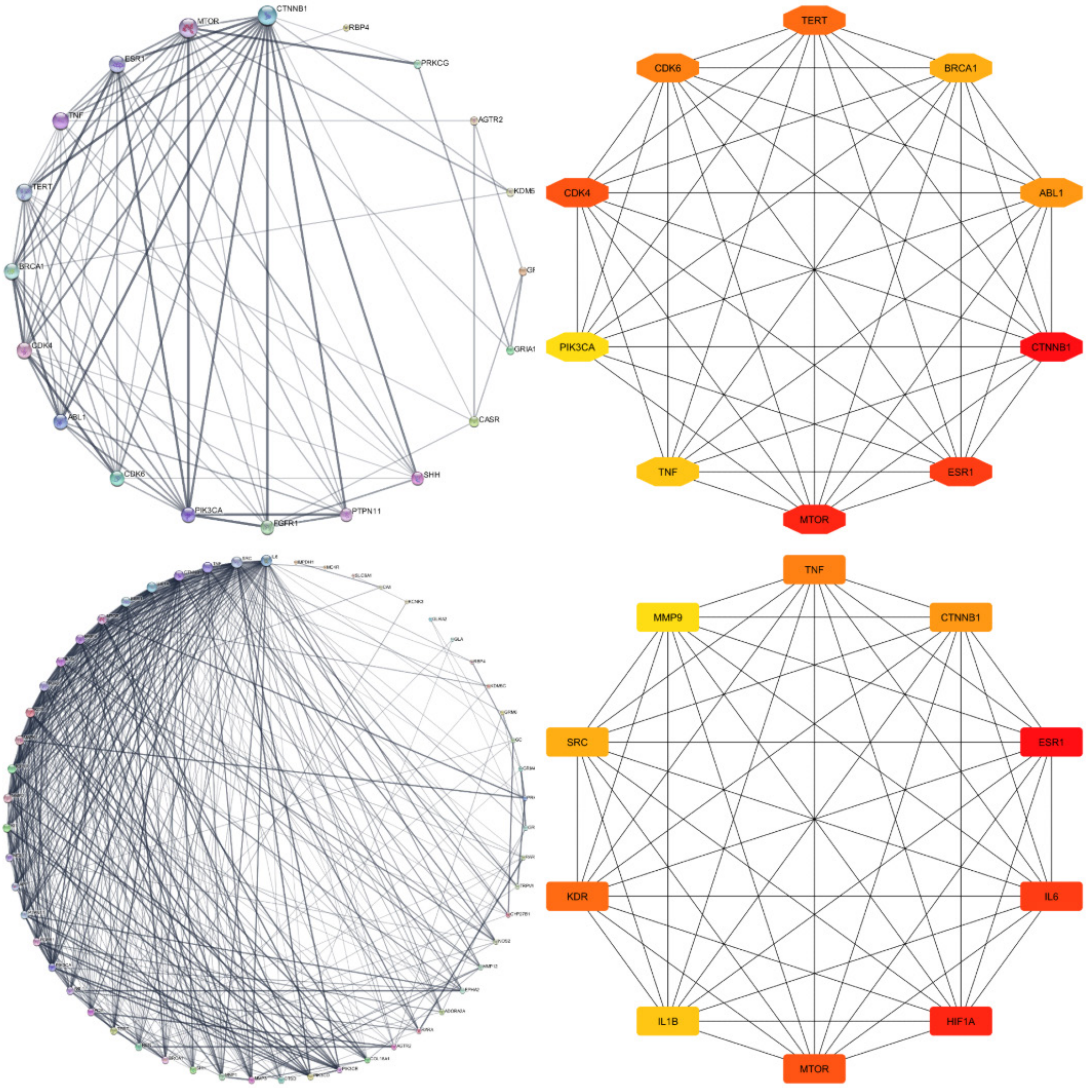


Figure 3: Above image is the PPI and Hub genes of Astigmatism and the below is the PPI and Hub genes of Myopia.

Table 2: Binding affinity of targets and ligand for "Astigmatism".

Phytochemicals	Binding Affinity (kcal/mol)		
	TNF	ESR1	CTNNB1
Liquiritin	-7.7	-9.3	-7.4
18-β-Glycyrrhetinic Acid	-6.8	-7.4	-8.2
Isoglycyrol	-7.3	-7.6	-7.6
Tetramethyl-pyrazine	-4.8	-5.7	-4.4

Table 3: Binding affinity of targets and ligand for "Myopia".

Phytochemicals	Binding Affinity (kcal/mol)		
	HIF1A	ESR1	IL6
Liquiritin	-7.1	-9.3	-6.9
18-β-Glycyrrhetinic Acid	-8	-7.4	-7.5
Isoglycyrol	-8.2	-7.6	-7.4
Tetramethyl-pyrazine	-4.3	-5.7	-4.5

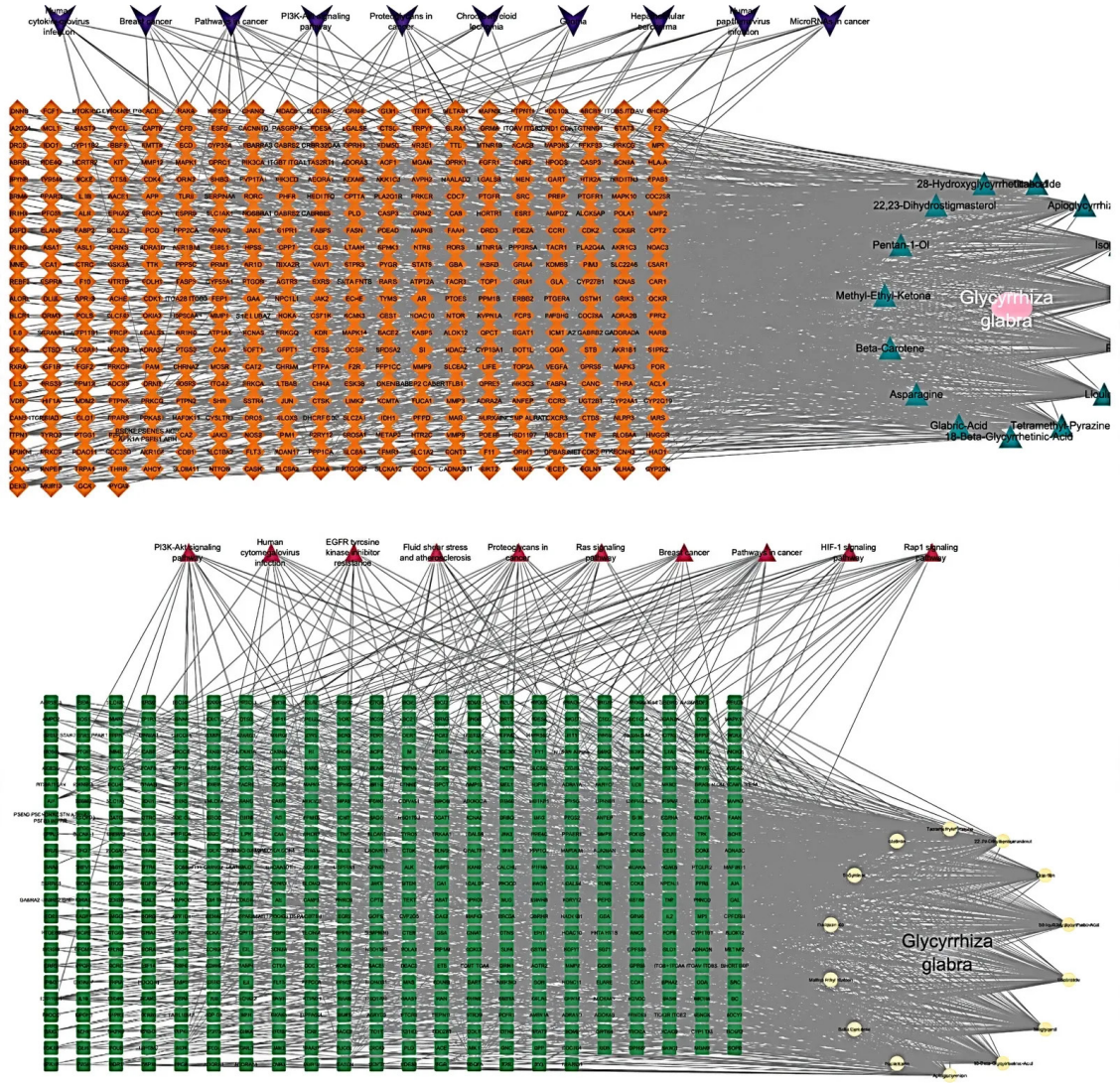


Figure 4: Topological analysis of Astigmatism is depicted above and Myopia is depicted below.

A study on *yastimadhu kalpa*, revealed that the physicochemical analysis had Water soluble extract value of 24.08%, while Alcohol soluble extract value was 13.23% and moreover the study's ash value was 4.88 (Chitrakar *et al.*, 2021). Usually if the water-soluble extract value is more in the plant then its interpretation is that there are more active bio-compounds, and so as to validate that the plant has more than 500 phytochemicals and secondary metabolites present in the root part as per the databases. And in a study on hydro-alcoholic extract study on *Glycyrrhiza* revealed that the anti-oxidant property of the leaf extract was performed and the DPPH scavenging activity of the extract's IC_{50} value was 385.85 $\pm$ 1.15 µg/mL, showing that the current study on the root has more anti-oxidant property in comparison to the leaf extract of the plant (Semenescu *et al.*, 2024). High oxygen demand, PUFA content, and light exposure all contribute to oxidative stress, which produces excess ROS that cause ocular cell death and age-related eye disorders. Carotenoids, anthocyanins, and vitamins are examples of antioxidant supplements that fight ROS and shield

retinal and lens cells. Although they have potential, substances like lutein, zeaxanthin, *saffron, bilberry*, and beta-carotene require additional clinical validation (Choo *et al.*, 2022).

Among the phytochemicals with highest degree layout liquiritin is one of the phytochemicals having a role on protective effect in eye tissues, the study revealed that liquiritin protects Human Corneal Endothelial Cells (HCECs) from apoptosis induced by an ophthalmic anaesthetic, by reducing reactive oxidative stress and inhibiting pro-apoptotic pathways (Li *et al.*, 2018). Moreover, studies on neuroprotective and anti-inflammatory reveal that the general cell protection under oxidative stress will also help in protecting ocular tissues also (Jia *et al.*, 2016). Though these studies reveal that they have potential role protection of eye tissues there are no studies that liquiritin improves eyesight.

Isoglycyrol is a flavonoid class that is present in *Glycyrrhiza glabra*, it is having the second highest binding affinity against the target. Flavonoid has a potential role in improving eyesight through preventing retinal pigment epithelium and delays lens opacity, improves blood flow in retinal vessels, protect endothelial cells, protects the optic nerve and retinal neurons mainly in diabetic retinopathy (Davinelli *et al.*, 2012).

ESR1 (Estrogen receptor 1) has a role on retinal pigment endothelium, human retina, ciliary body and iris. The expression of the genes varies according to the age and gender (Ogueta *et al.*, 1999). A review on estrogenic retina states that sex-hormone signalling may influence retinal ageing, susceptibility to ocular diseases especially around menopause and hormonal changes (Cascio *et al.*, 2015). Thus, this suggests that the target has a role in the disease. If the receptor is maintained normally then there is a chance that it may lower the retinal aging and ocular diseases.

According to a recent study on experimental myopia in guinea pigs, PI3K/Akt signaling is disrupted in scleral remodeling, resulting in axial elongation and scleral thinning due to alterations in apoptosis/cell-adhesion indicators, decreased collagen I, decreased TIMP-2, and elevated MMP-2 (Li *et al.*, 2025). Refractive error and myopia risk are linked to variations or methylation changes in genes of the Beta-catenin pathway, extracellular matrix/collagen genes, and other developmental pathways, according to extensive human genetic and epigenetic studies (Vishweswaraiah *et al.*, 2019).

The molecular docking analysis gave highest binding affinity with liquiritin towards ESR1, followed by that HIF1A towards Isoglycyrol. Liquiritin had the highest average binding affinity of -9.23 kcal/mol and Tetramethyl pyrazine had the lowest average binding affinity of -4.9 kcal/mol. Though, liquritin had favourable hydrogen bonding towards LEU: 328, ARGA: 394 it had unfavourable hydrogen bonding towards LEU: 525, while

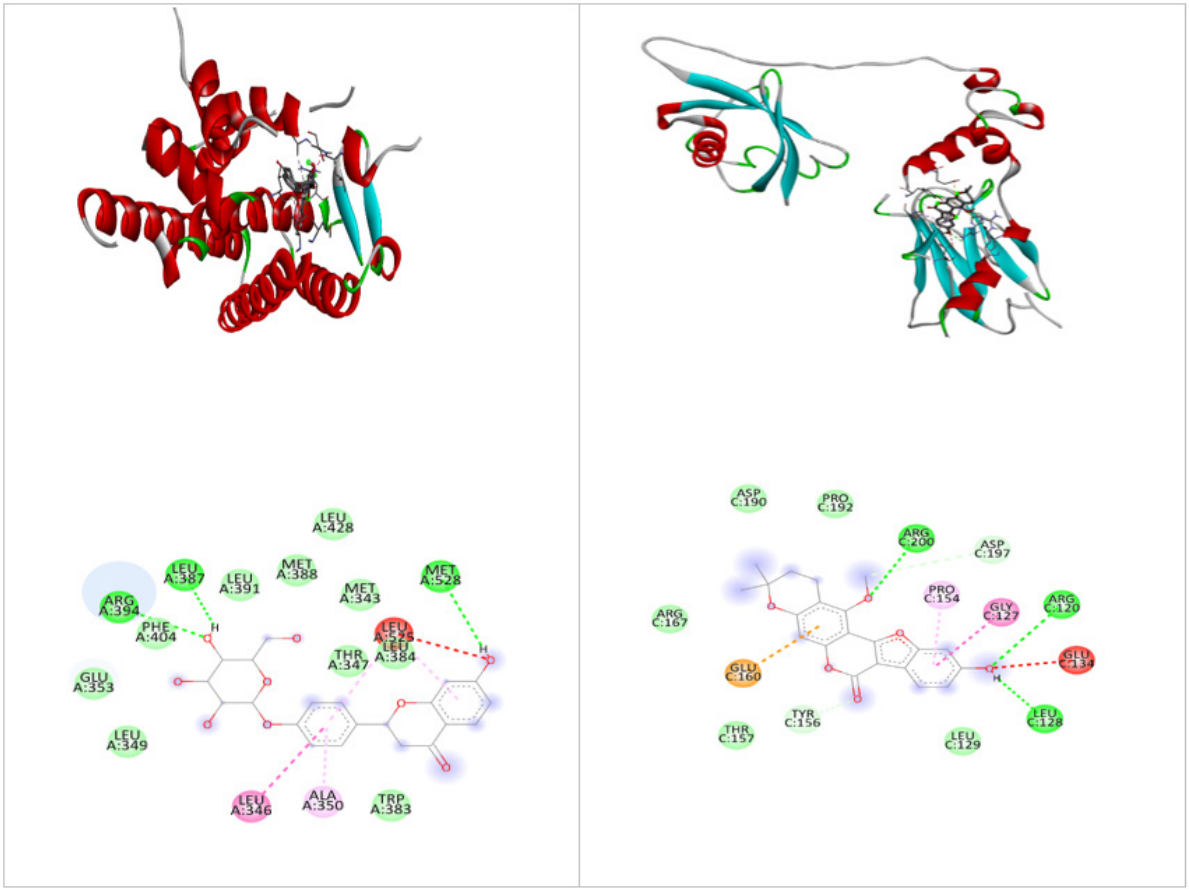


Figure 5: Molecular docking analysis of Liquiritin and ESR1 on the left while Isoglycyrol and HIF1A in the right side.

in isoglycyrol had one unfavourable hydrogen bonding towards GLUC: 134. Even isoglycyrol had 3 favourable hydrogen bonding making a strong ligand and target interaction.

Even though these findings indicate that the target, compound, and pathway have an impact on refractive errors through oxidative stress, degeneration, or apoptotic pathways, there is still insufficient evidence to conclude that these factors directly contribute to refractive errors, which opens up new avenues for research on the compounds and target. Aside from this, the study has a flaw in that it only measured antioxidant activity rather than phytochemical analysis and did not quantify the major phytochemical classes. The compounds used in the *in silico* study were obtained from databases rather via spectroscopic examination, raising doubts about the study's dependability because the databases are periodically updated.

CONCLUSION

The medicine based on plants has been used since centuries in curing many major diseases, one among being refractive errors. *Yastimadhu* though it is used as a medicine since ages in refractive disorders, the mechanism behind its usage was still unclear. Hence while performing the anti-oxidant activity validated the role of the plant in reducing the ROS and further while performing *in silico* activity it revealed more overlapping targets between Myopia and *Yastimadhu* with a total target of 58. Moreover, in the disease myopia there were 135 pathways and the topological analysis revealed 10 phyto-compounds with highest degree layout. Upon literature review 4 compounds had possible role on refractive disorders, further on ligand and target interaction in molecular docking discovered the role of liquritin and isoglycyrol in ESR1 and HIF1A respectively. Additionally, studies on *in vivo* and clinical evaluation on human participants will validate the ancient wisdom with modern parameters.

ABBREVIATIONS

API: Ayurvedic Pharmacopoeia of India; **GMP:** Good Manufacturing Practice; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **IMPPAT:** Indian Medicinal Plants, Phytochemistry and Therapeutics; **SMILES:** Simplified Molecular Input Line Entry System; **QED:** Qualitative Estimation of Drug-likeliness; **HIA:** Human Intestinal Absorption; **HPA:** Human Protein Atlas; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **PPI:** Protein-Protein Interaction; **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity; **RCSB PDB:** Research Collaboratory for Structural Bioinformatics Protein Data Bank; **IC_{50}:** Half maximal inhibitory concentration; **ROS:** Reactive Oxygen Species; **PUFA:** Polyunsaturated Fatty Acids; **HCEC:** Human Corneal Endothelial Cell.

AUTHORS CONTRIBUTION

KS: Investigation, Methodology, Writing-original draft, SKS: Conceptualization, Supervision, Project administration, AB, SS: Data curation, Formal analysis, Validation, SVKV: *In silico* analysis and drafting.

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