



***In silico* Analysis of Phytochemicals from *Glycyrrhiza glabra* against Conjunctivitis**

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SS, SG, SD and DB conceived and planned the study, while author SS performed the computational framework and analyzed the statistical data. Author SD carried out the implementation. Authors SS and SG wrote the first draft of the manuscript. Authors SS, SG and SD managed the analyses of the study. Author SD took lead in the literature searches. Author MP read and approved the final manuscript and provided assistance throughout the study, while author DB supervised and guided the whole study.

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ABSTRACT

Phytochemicals are naturally occurring biologically active compounds found in plant parts such as roots, barks, leaves, seeds, and even pulps. It has been reported that *Glycyrrhiza glabra* plant extract is used for treatment of the disease conjunctivitis. The plant extract contains different phytochemicals. The agent that causes conjunctivitis is a gram positive bacterium which belongs to species *Staphylococcus*. One of the key enzymes involved in its biochemical pathway is Isocitrate dehydrogenase. The molecular docking of the phytochemicals with the enzyme was studied using Biovia Discovery Studio. The strength of the interaction was evaluated based on -CDocker energy and -CDocker interaction energy. High positive values for both the parameters indicated that out of different phytochemicals rosmarinic acid can effectively deactivate the isocitrate dehydrogenase enzyme thereby inhibiting the life cycle of *Staphylococcus*.

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1. INTRODUCTION

Since prehistoric times, the treatment and cure of diseases has been one of the primary concerns of mankind. Local practitioners have used indigenous plants and herbs for centuries all over the world to treat a variety of ailments and these have exhibited clear pharmacological activities [1]. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and presumably lesser side effects. Plant derivatives had been employed by population to prevent different kind of diseases for centuries. The knowledge of plant properties was acquired by ancient civilization that passed down from generation to generation until today. Plant showed wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardiovascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic antipyretic and many other pharmacological effects [2]. Plants and their secondary metabolite constituents have a long history of use in modern western medicine and in certain systems of traditional medicine and are the sources of important drugs such as atropine, codeine, digoxine, morphine, quinine and vincristine etc. Use of herbal medicine in developed countries has expanded sharply in the latter half of twentieth century. The pharmacological treatment of disease began long ago with the use of herbs [3]. The medicinal value of the plants lies in some chemical substances that produce a definite physiological action on the human body, these substances are called phytochemicals, which can be used for therapeutic purpose. Various parts of plants like bark, leaves, flowers, roots, exudates etc. are used as per medicinal properties. Plants containing beneficial phytochemicals may supplement the needs of the human body by acting as natural antioxidants [4]. Various medicinal plants and their phytoextracts have shown numerous medicinal properties like antioxidant, anti-inflammatory, anti-cancer, antimicrobial, anti-diabetes action etc. Medicinal plants play a crucial role in human health care and wellness program. The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare, which is predominantly

based on plants, the reasons of this popularity are the safety, promising potential with efficacy of medicinal plants and their cost effectiveness [5]. In the developed countries, the most important among many other reasons for seeking herbal therapy is the belief that it will promote healthier living. Herbal medicines are, therefore, often viewed as a balanced and moderate approach to healing and individuals who use them as home remedies and over-the-counter drugs spend huge amount of money (in excess of billions of dollars) on herbal products [6]. Many of the medicinal plants are used as spices and food items. They also played an important role in many medicines like allopathic medicine, herbal medicine, alternative medicine, homoeopathy and aromatherapy [7]. Among different sources of natural products, plants have been a source of novel chemical substance, which serves as starting materials for a number of old and new pharmaceutical products.

Medicinal plants are the foundation of many drugs prescribed today in modern medicinal system. About 25% of modern pharmaceutical drugs have botanical origins, for example, the herb foxglove is the source for digitalis and the herb salicin is the source for aspirin. The breast-cancer-fighting drug taxol (tamoxifen) comes from the pacific yew tree, quinidine from *Cinchona* spp., vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum* [8]. Research needs in the field of medicinal plants are huge, but are balanced by the potential health benefits and the enormous size of the market. Research into the quality, safety, biological activity, and clinical efficacy of the numerous plants in common usage is required. Newly emerging scientific techniques and approaches have been used in the growing area of medicinal plant research, for the investigation of constituents and determination of biological activity of medicinal plants. Evidence for the beneficial effects of selected plants is generally based on experiments demonstrating a biological activity in a relevant in vitro bioassay or experiments using animal models [9]. Plants that demonstrated anticancer, antioxidant, anti-inflammatory, immune stimulatory and antimicrobial properties have received research attention.

Mulethi belongs to family *Fabaceae*. Mulethi leaves extract is used to cure diseases like

conjunctivitis. The phytochemical screening of the *Glycyrrhiza glabra* root revealed the presence of alkaloids, glycosides, carbohydrates, starches, phenolic compounds, flavonoids, proteins, pectin, mucilage, saponins, lipids, tannins, sterols and steroids. Mulethi is known to contain phytochemicals like phloretin, rosmarinic acid, Isocoumarin, Cortison, Tanic acid, Liquiritigenin, Harmalol, Glycyrrhizin, Alpha-terpene etc. and so on [10]. There is high possibility that these phytochemicals play a major role in curing conjunctivitis. However, there is no report identifying the specific phytochemical responsible to cure conjunctivitis.

A group of bacteria belonging to genus *Staphylococcus* generally causes conjunctivitis. They are Gram-positive bacteria cocci. *Staphylococcus* infections may cause disease due to direct infection or due to the production of toxins by the bacteria. Symptoms and signs of a localized staph infection include a collection of pus, such as a boil, furuncle, or abscess. The area is typically tender or painful and may be reddened and swollen [11]. *Staphylococcus* infection is a common bacterial disease that affects the eyelids. *Staphylococcus* bacteria typically live in animal and human skin and are shed inside the nostrils [12]. Humans become infected most frequently through cuts, abrasions, skin to skin contact [13].

This study focuses on the identification of the phytochemical of *Glycyrrhiza glabra* responsible to cure conjunctivitis caused by *S. aureus*.

2. MATERIALS AND METHODS

2.1 Software Used

Discovery studio module of Biovia software (Dassault Systems of France) was used for analysis. The software utilizes machine learning techniques to predict the level of molecular interaction and determines the three-dimensional structure and properties of a macromolecule.

2.2 Methodology

2.2.1 List of phytochemicals

Phytochemicals are produced by plants as secondary metabolites to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some phytochemicals have been used as poisons and

others as traditional medicine. Published works showed that *Glycyrrhiza glabra* contains phloretin, rosmarinic acid, Isocoumarin, cortison, tannic acid, liquiritigenin, harmalol, glycyrrhizin, alpha-terpene etc. It has already been established that *Glycyrrhiza glabra* plant belonging to *Fabaceae* family has potential to help controlling conjunctivitis. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of conjunctivitis.

2.2.2 Enzyme found in *Staphylococcus*

It has been reported that conjunctivitis can be caused as a result of *S. aureus* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Salmonella aureus* bacteria. It has been found that isocitrate dehydrogenase enzyme (protein database code 2BOT) is involved in (lysine metabolism) and very crucial for survival of the particular microbe.

2.2.3 Molecular docking

Molecular docking method has been used to identify the phytochemical from the plant extract that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The Discovery studio module of Biovia software was used for identifying molecular interaction and performs molecular docking. In this process first the sdf files for the phytochemicals found in the *Glycyrrhiza glabra* plant were downloaded from the website. The protein database code of the isocitrate dehydrogenase enzyme was identified from the website. The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDocker protocol of Biovia software under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY" and "-CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of molecular docking. The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

3. RESULTS AND DISCUSSION

Fig. 1 shows the active site of the isocitrate dehydrogenase enzyme. It appears as light green colour. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based molecular docking method and optimized for accuracy. The ligand conformations were obtained by Molecular Dynamic methods.

-CDOCKER energy was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction

signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on a) high positive value of -CDOCKER energy and b) small difference between -CDOCKER energy and -CDOCKER interaction energy (OP Brinda¹, Deepu Mathew², MR Shylaja¹, P Sangeetha Davis³, K Anita Cherian⁴, PA Valsala, Isovaleric acid and avicequinone-C are Chikungunya virus resistance principles in *Glycosmispentaphylla* (Retz.) Correa, 2019, 56(2), 111-121). Table 1 show that isocitrate dehydrogenase rosmarinic

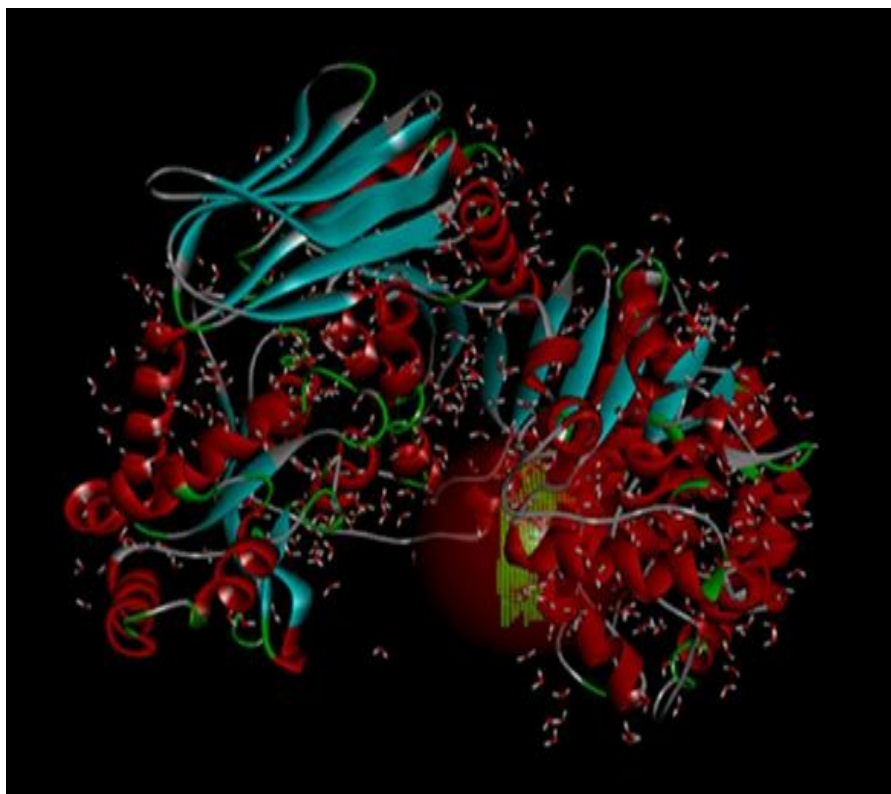


Fig. 1. Active site of glycerol dehydrogenase enzyme

Table 1. Results of CDocking of phytochemicals with Isocitrate dehydrogenase (receptor)

SI no	Ligand	-C DOCKER energy	-C DOCKER interaction energy	Difference between -C DOCKER interaction energy and -C DOCKER energy
1	Rosmarinic acid	31.7015	29.3129	-2.3886
2	(+)-catechine	22.3665	29.7124	7.3459
3	Alpha-terpineol	-10.6396	17.5426	28.1822
4	Geraniol	-22.9813	21.8162	44.7975
5	Harmalol	8.93449	20.5686	11.63411
6	Tanic acid	Failed	Failed	NA

acid interaction has the highest positive value of -CDOCKER energy 31.7015 and minimum value of the difference -2.3886 between -C DOCKER interaction energy and -C DOCKER energy followed by catechine. Thus, the results indicated that rosmarinic acid and catechine can effectively deactivate the isocitrate dehydrogenase enzyme thereby interrupting the biological cycle of *S. aureus*. Higher positive values for rosmarinic acid indicated that it was the most active ingredient against *S. aureus*. On the other hand alpha-terpineol, Geraniol, Harmalol can deactivate the enzyme to a small extent (negative -CDOCKER energy but positive -CDOCKER interaction energy). Tanic acid cannot interact with Isocitrate dehydrogenase enzyme. Thus, the key phytochemicals preventing conjunctivitis caused by *S. aureus* are rosmarinic acid and catechine.

4. CONCLUSION

It has been previously reported that *Glycyrrhiza glabra* plant has medicinal action against conjunctivitis. Conjunctivitis is caused by *Staphylococcus* sp. A systematic study has been undertaken to provide the theoretical basis of this observation. Molecular docking operation was performed using Discovery studio module of Biovia software, in order to identify and evaluate the phytochemical (phloretin, rosmarinic acid, Isocoumarin, Cortison, Tanic acid, Liquiritigenin, Harmalol, Glycyrrhizin, Alpha-terpene etc.), and these phytochemicals have a significant interpreting interaction with the vital enzyme isocitrate dehydrogenase of the pathogenic microorganism. It was found that rosmarinic acid and catechine can form strong bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Alpha-terpineol, Geraniol, Harmalol were found to be not much effective in deactivating the enzyme of the microbe. Tanic acid cannot deactivate the enzyme. Thus, according to the results the presence of rosmarinic acid and catechine provided the medicinal values to *Glycyrrhiza glabra* against conjunctivitis caused by pathogenic *Staphylococcus aureus*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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