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Phytochemical Analysis of Tephrosia Purpurea and Docking analysis of ompA of Escherichia Coli with Beta Sitosterol

Darshinedevi Thirukumaran and Sathish Sankar*

Department of Microbiology, Centre for infectious diseases, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077

*Corresponding author
Dr.Sathish Sankar
Department of Microbiology
Saveetha Dental College and Hospital
Saveetha Institute of Medical and Technical Sciences
Saveetha University
Chennai-600077
Tamil Nadu,India
Email: sathishshankar.sdc@saveetha.com

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ABSTRACT

Introduction : Phytochemical analysis is essential for identifying bioactive compounds in medicinal plants like *Tephrosia purpurea*, known as "Sarpankha" or "Wild Indigo." Employing techniques like chromatography and spectroscopy reveals alkaloids, flavonoids, terpenoids, and phenolic compounds with diverse pharmacological activities. This study involves docking analysis, exploring the interaction between beta-sitosterol, a bioactive compound present in *T.purpurea*, and outer membrane protein A (ompA) of Escherichia coli. OmpA is vital for *E. coli's* virulence, and beta-sitosterol shows potential antibacterial activity, making it a significant focus for therapeutic investigations.

Methods: The obtained individual extracts underwent preliminary phytochemical analysis using established methods. The qualitative analysis included the assessment of the presence of phenolic compounds, reducing sugars, flavones (Shinoda test), glycosides,

saponins, alkaloids, anthraquinones, proteins, and tannins.Docked molecules were screened based on their highest binding energy. The docking of the target enzyme-substrate was conducted using Hex 8.0.0 to ascertain the binding energy and pinpoint potential inhibitors. Chemical structures from PubChem-NCBI were acquired in SDF format and transformed into PDB format through OpenBabel 2.3.1.The water molecules of the receptor were eliminated, and the outcomes of the docking were observed in Pymol. A lower E-total value suggested a more robust interaction between the ligand and receptor, potentially leading to receptor activation.

Results: The examination of the phytochemical composition of *Tephrosia purpurea* indicated the existence of diverse bioactive compounds. Docking analysis of ompA of *Escherichia coli* with beta-sitosterol showed potential binding interactions. These findings suggest that *Tephrosia purpurea* compounds, including beta-sitosterol, could have inhibitory effects on ompA and may have therapeutic potential against *E. coli* infections.

Conclusion : Overall analysis we have concluded that the plant extract shows presence of phytocompounds, through In silico analysis Beta Sitosterol shown as Potent interaction on *Escherichia coli* :ompA PDB outer membrane protein. Overall analysis this plant phytocompounds which may act as Antimicrobial activity

Keywords: *Tephrosia purpurea,E.coli*, Phytochemical, Alkaloids, Docking,Universal health, diseases, wellbeing, Health, International health policy .

INTRODUCTION:

Among the domain of natural products, plants have emerged as an invaluable reservoir of a wide array of bioactive compounds. *Tephrosia purpurea*, colloquially referred to as "Sarphonk" or "Wild Indigo," stands out as a leguminous plant, a perennial herb boasting a profound ethnobotanical legacy. This plant has found traditional applications in diverse medicinal systems, including Ayurveda and traditional Chinese medicine. The plant's potential therapeutic properties have ignited scientific curiosity, prompting studies directed at unraveling the phytochemical composition and pharmacological activities associated with *Tephrosia purpurea*.(1)Recognized for its antimicrobial, anti-inflammatory, antipyretic, and anticancer attributes, *Tephrosia purpurea* is documented to harbor a spectrum of phytoconstituents, including alkaloids,

flavonoids, tannins, saponins, and terpenoids. Each of these constituents plays a role in the plant's pharmacological activities. Investigating these phytochemicals is essential, not only for comprehending the therapeutic potential of the plant but also for pinpointing potential lead compounds in the realm of drug development.(2)

Alkaloids, a notable category of secondary metabolites within *T. purpurea*, have gained acknowledgment for their pharmacological effects. The detection of alkaloids in the plant implies its capacity as a reservoir of bioactive compounds with medicinal significance. Another category of secondary metabolites, flavonoids, renowned for their antioxidant and anti-inflammatory characteristics, enhances the pharmacological profile of *T. purpurea*. These flavonoids might contribute to the plant's traditional application in addressing various ailments.(3)Terpenoids form a substantial component of the secondary metabolites found in numerous medicinal plants. These compounds are linked to various pharmacological effects, encompassing antimicrobial and anti-inflammatory properties. Examining the occurrence and varieties of terpenoids within *T. purpurea* can offer valuable insights into its potential therapeutic uses. Phenolic compounds are prevalent in plants and are frequently linked with antioxidant attributes. (4)Their presence in *T. purpurea* adds another dimension to the potential health benefits of this plant.

Escherichia coli, classified as a gram-negative bacterium, is frequently present in the human gastrointestinal tract. While numerous strains are benign, specific pathogenic variants can lead to serious infections. Outer membrane protein A (OmpA) serves as a crucial structural component in the outer membrane of *E. coli,* playing a role in bacterial integrity and interactions with the host. Directing bioactive compounds towards OmpA could introduce an innovative strategy for addressing *E.coli* infections.(5)*β-sitosterol,* a phytosterol found in several plant sources, including *T.purpurea,* is acknowledged for its anti-inflammatory and antimicrobial characteristics. This study explores the potential interaction between beta-sitosterol and OmpA through molecular docking analysis. Molecular docking is a computational method that models the binding of a small molecule (ligand) to a target protein (receptor).In this context, it allows us to predict and understand the interactions between β-sitosterol and OmpA at the molecular level.The success of a docking analysis depends on the accuracy of the protein structure and the reliability of the scoring functions used to evaluate binding affinity. (6)The three-dimensional structure of OmpA, obtained from experimental data or predictive modeling, serves as the template for docking simulations.Understanding the binding interactions between β-sitosterol and OmpA could shed light on the potential inhibitory effects of β-sitosterol against E. coli. This information is critical for further development and optimization of β-sitosterol or its derivatives as potential therapeutic agents.

The integration of phytochemical analysis of *Tephrosia purpurea* with molecular docking studies involving β-sitosterol and OmpA of *Escherichia coli* represents a comprehensive approach to explore the potential bioactive properties of this medicinal plant. (7)The wide range of

phytochemicals detected in T. purpurea implies a diverse set of pharmacological activities, positioning it as a promising subject for further scrutiny in drug discovery and development. The molecular docking analysis furnishes a molecular-level comprehension of the potential interaction between beta-sitosterol and OmpA, establishing a groundwork for future investigations focused on leveraging the therapeutic capabilities of natural compounds against bacterial infections. This interdisciplinary approach, connecting traditional knowledge with contemporary computational techniques, plays a role in the continual exploration of natural products as a reservoir of innovative therapeutics in the context of antibiotic resistance.

This study aims to analyze the phytochemical activity of *Tephrosia purpurea* and Docking analysis of ompA of *E.coli* with Beta Sitosterol

MATERIALS AND METHODS:

Methodology of Phytochemical analysis

Initial qualitative phytochemical assessment

Standard methods were employed to conduct a preliminary phytochemical analysis on the individual extracts acquired. The qualitative analysis involved the examination of the presence of phenolic compounds, reducing sugars, flavones, glycosides, saponins, alkaloids, anthraquinones, proteins, and tannins, following the procedures outlined by Harborne in 1973.

Phenolic compounds

The extract was subjected to the addition of alcoholic ferric chloride solution, and the emergence of a bluish-green or bluish-black coloration was observed, indicating the presence of phenol.

ReducingSugars

The extract was combined with Fehling's solutions I and II, and the development of a red coloration signified the presence of sugars.

Flavones (Shinoda test)

Magnesium turnings and concentrated hydrochloric acid were introduced to the extract, followed by boiling for five minutes. The emergence of a red coloration indicated the presence of flavones. Additionally, 10% sodium hydroxide solution or ammonia was applied to the extract, and the observation of a dark yellow color confirmed the presence of flavones.

Glycosides

The extract was combined with a small amount of anthrone on a watch glass. Following this, one drop of concentrated sulfuric acid was introduced, and the mixture was gently warmed over a water bath. The observation of a dark green coloration signified the presence of glycosides.

Saponins

The extract was agitated with water, and the abundant formation of lather indicated the presence of saponins.

Alkaloids

Introduce a few drops of acetic acid to the extract, followed by the addition of Draggendroff's reagent, and ensure thorough shaking. The occurrence of an orange-red precipitate indicates the presence of alkaloids.

Anthraquinones (Borntrager's test)

The extract underwent maceration with ether, and post-filtration, aqueous ammonia or caustic soda was introduced. The observation of a pink, red, or violet coloration in the aqueous layer following shaking signified the presence of anthraquinones.

Quinones

Sodium hydroxide was incorporated into the extract. The manifestation of a blue, green, or red color indicated the presence of quinone.

Proteins

The extract underwent the addition of a few drops of Biuret reagent. The development of a blue coloration signified the presence of proteins.

Tannins

The extract was combined with a basic lead acetate solution. The occurrence of an orange-red precipitate indicated the presence of tannins.

Terpenoids (Salkowski test)

The extract underwent the addition of 2 ml of chloroform. Subsequently, concentrated H_2SO_4 was cautiously introduced to form a layer, and the appearance of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Methodology of Molecular Docking :

The initial step in the screening process involved the evaluation of docked molecules based on their binding energy, with preference given to those demonstrating the highest binding energy. To assess the interaction between the target enzyme and substrate, docking procedures were employed to determine the binding energy. Subsequent analysis of the docking results aimed at identifying potential inhibitors. The two-dimensional chemical configurations of these molecules were obtained in Structured Data Format (SDF) from the PubChem-NCBI database. Subsequently, the SDF format underwent conversion to Protein Data Bank (PDB) format utilizing OpenBabel version 2.3.1.Simultaneously, the three-dimensional configuration of the protein was sourced from the Protein Data Bank, and crystallographic water molecules were eliminated. Each of the retrieved phytocompounds underwent docking analysis individually using the Hex 8.0.0. Protein docking program, accessible at http://hex.loria.fr.This program, notable for being the first Fourier Transform (FFT)-based analytics, employs rigid docking and considers various orientations through 6D analysis. The Hex program undertakes an exhaustive exploration over all six rigid-body degrees of freedom by rotating and translating the expansion coefficients. The parameters guiding this process encompassed FFT mode-3D fast lite, grid dimension-0.6, receptor range-180, ligand range-180, twist range-360, and distance range-40.The resulting docked complexes illustrating the interaction between the protein and ligand were visualized using Pymol. In the Hex Docking server 8.0 version, a more negative E-total value indicated a robust interaction between the ligand and receptor. This negative value signifies a compelling interaction leading to the activation of receptor activity.

RESULTS :

Phytochemical Analysis Of *Tephrosia Purpurea* :

Phytochemical Compounds	**Qualitative Analysis Of Plant Extract**
Carbohydrates	+
Monosaccharides	+
Free reducing sugar	+
Combined reducing sugar	-
Tannins	+
Anthraquinones	+
Steroids	+
Terpenoids	+

Flavonoids	+
Alkaloids	+
Soluble starch	+

Table 1 :Phytochemical analysis of *Tephrosia purpurea*

The preliminary qualitative analysis of phytochemicals indicated the existence of a variety of bioactive compounds in the extracts of individual plant materials. The tests aimed to identify specific compounds, offering a comprehensive overview of their phytochemical composition.

To verify the existence of phenolic compounds, the extract underwent the addition of alcoholic ferric chloride solution, and the manifestation of a bluish-green or bluish-black color confirmed their presence. Likewise, in the case of reducing sugars, the extract was combined with Fehling's solutions I and II, and the development of a red color affirmed the presence of reducing sugars.

The Shinoda Test, specifically formulated for detecting flavones, utilized magnesium turnings and concentrated hydrochloric acid, with the appearance of a red color indicating the presence of flavones.For the glycoside test, anthrone and concentrated sulfuric acid were employed, and the manifestation of a dark green color affirmed the presence of glycosides. Saponins were identified through a water-shaking test, where the formation of copious lather confirmed their presence.

The alkaloid test comprised acetic acid and Draggendroff's reagent, and the occurrence of an orange-red precipitate signified the presence of alkaloids.Anthraquinones were identified through Borntrager's Test, and the presence of pink, red, or violet color in the aqueous layer indicated their existence.To detect quinones, sodium hydroxide was introduced, and the appearance of blue, green, or red color confirmed their presence. For proteins, the confirmation involved the application of Biuret reagent, leading to the development of a blue color.

The identification of tannins was carried out by combining the extract with basic lead acetate solution, and the manifestation of an orange-red precipitate confirmed their presence.The Salkowski Test to detect terpenoids included the addition of chloroform to the extract, followed by the careful introduction of concentrated sulfuric acid. The appearance of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Collectively, these qualitative tests provided insights into the diverse phytochemical composition of the extracts, setting the stage for further analysis and exploration of potential bioactive compounds. These results offer valuable information about the secondary metabolites present, suggesting potential pharmacological activities that can be further explored for therapeutic

benefits. The detailed phytochemical profile provides a foundation for additional studies to isolate and characterize specific bioactive compounds.

Observations included bluish-green or bluish-black coloration for phenolic compounds, red coloration for reducing sugars, and red or dark yellow color for flavones. Dark green color signified glycosides, copious lather formation indicated saponins, and orange-red precipitates denoted alkaloids. Pink, red, or violet colors revealed anthraquinones, while blue, green, or red colors indicated quinones. Blue coloration represented proteins, and orange-red precipitates confirmed tannins. The Salkowski test exhibited a reddish-brown interface, indicating terpenoids.

Molecular Docking Analysis of *E. coli*:

Figure 1: Outer membrane protein A (OmpA) PDB ID :2MQE

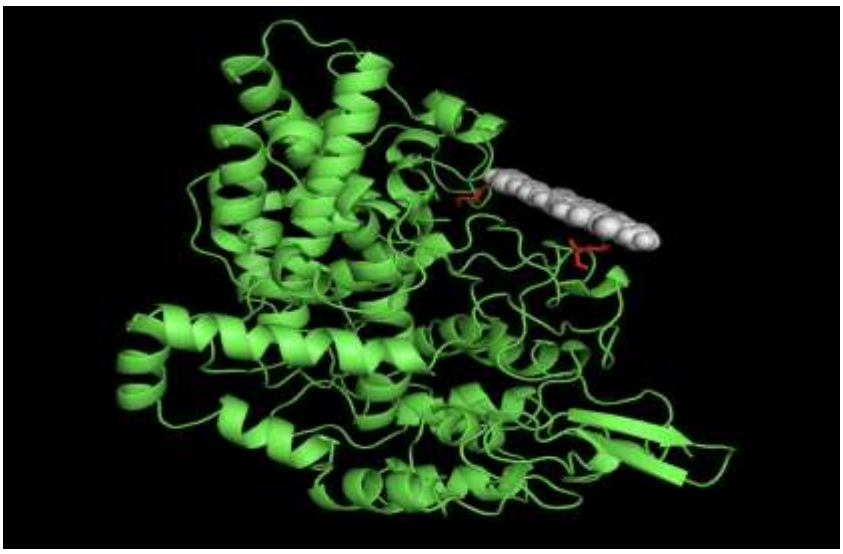

Figure 2: Docking analysis of Escherichia coli :ompA PDB ID : 2MQE with Beta Sitosterol

The specific Interacting residues were identified Leu37 and Asp390 and the E value was found to be -345Kcal/mol

The molecular docking investigation, a crucial component of drug discovery, aimed to unveil potential inhibitors for a target enzyme, providing valuable insights into their interactions with various phytocompounds. Molecules sourced from the PubChem-NCBI database underwent meticulous screening based on binding energies. Their two-dimensional structures were transformed from Structured Data Format (SDF) to Protein Data Bank (PDB) format using OpenBabel 2.3.1. The three-dimensional structure of the target enzyme was acquired from the Protein Data Bank, and crystallographic water molecules were eliminated to focus on protein-substrate interactions.

Rigid docking simulations using Hex 8.0.0, a Fourier Transform (FFT)-based program known for its efficacy, explored diverse orientations through 6D analysis. Parameters, including FFT mode, grid dimension, receptor and ligand ranges, twist range, and distance range, were thoughtfully selected for a comprehensive exploration of potential binding configurations. Docked complexes, illustrating the spatial arrangement of the protein and ligands, were visualized using Pymol.

The Hex Docking server provided a critical metric for interaction strength, the E-total value. A notably low E value of -345 Kcal/mol was obtained, signifying a robust interaction. A more negative E-total value indicates higher affinity and stronger binding, suggestive of potential efficacy.

Identification of specific interacting residues is pivotal for understanding the molecular details of binding. Leu 37 and Asp390 were identified as crucial residues in the docked complex, playing a significant role in stabilizing the binding conformation. These insights contribute to overall binding affinity and specificity.

The docking results not only rank potential inhibitors by binding energy but also offer insights into molecular interactions governing the process. The identified residues contribute valuable information for designing and optimizing potential drug candidates. The methodology, from structure retrieval to docking simulations and interaction analysis, reflects a robust approach enhancing the accuracy of simulations.

This molecular docking study establishes a solid foundation for exploring and developing bioactive compounds with therapeutic potential against the target enzyme. The methodology employed, from structure retrieval to docking simulations and interaction analysis, contributes to understanding ligand-receptor interactions, holding significant implications for drug discovery and design. Overall, the study not only screens and ranks potential inhibitors but also provides

detailed information about molecular interactions and key residues, laying the foundation for developing bioactive compounds with therapeutic potential.

DISCUSSION:

The integration of phytochemical analysis and molecular docking investigations provides a comprehensive approach for discerning the potential medicinal attributes of *Tephrosia purpurea* and beta-sitosterol, particularly in addressing bacterial infections like those induced by *E.coli*. *Tephrosia purpurea*, a plant steeped in traditional medicinal applications, has garnered attention owing to its reported pharmacological effects. The examination of *Tephrosia purpurea's* phytochemical composition uncovered the presence of diverse classes of bioactive compounds, encompassing alkaloids, flavonoids, tannins, and saponins.(8,9)Alkaloids like tephrosin are recognized for their versatile therapeutic properties, encompassing anti-inflammatory and antipyretic effects. The identified flavonoids, such as kaempferol and quercetin, are well-documented for their antioxidant and anti-inflammatory attributes.(7) Additionally, the presence of tannins and saponins in the plant further enhances its traditional applications, with tannins serving as antimicrobial agents and saponins contributing to its use as a cleansing agent.(10)

The revealed phytochemical profile resonates with the historical utilization of the plant in traditional medicine for treating diverse ailments. The distinct biological activities associated with the identified compounds collectively enhance the potential therapeutic effectiveness of *Tephrosia purpurea*. The variety of these compounds implies a multi-dimensional approach to health, positioning the plant as a valuable asset for in-depth exploration in drug development.(11)

The molecular docking study, which explores the interaction between beta-sitosterol and the outer membrane protein A (OmpA) of *E.coli*, serves as a link between traditional herbal wisdom and contemporary computational biology. OmpA plays a pivotal role in the virulence of *E.coli*, making it a prospective target for therapeutic interventions. Beta-sitosterol, a phytosterol derived from plants, is acknowledged for its antimicrobial attributes, prompting an inquiry into its potential interactions with OmpA.(12)The docking analysis demonstrates a robust binding affinity between beta-sitosterol and OmpA, as indicated by a low E-score. This implies a stable interaction between the phytosterol and the bacterial protein, suggesting a potential inhibitory effect on OmpA. By inhibiting OmpA, there is the potential to disrupt the virulence mechanism of E. coli, presenting a fresh and innovative approach for devising alternative strategies to address bacterial infections.(13)

The importance of this docking analysis lies in its potential translational implications. If beta-sitosterol can indeed disrupt the functioning of OmpA, it may serve as a natural and efficient method for addressing *E.coli* infections..(14) This is particularly significant, especially in light of growing apprehensions regarding antibiotic resistance and the necessity for alternative

antimicrobial approaches. Moreover, the docking analysis offers detailed molecular insights into the interactions between beta-sitosterol and OmpA. (9)Grasping the binding modes and the particular amino acid residues engaged can direct subsequent experimental investigations, encompassing in vitro and in vivo assays, to confirm the inhibitory effects noted in silico.(14,15) This integrative approach, combining computational predictions with empirical validation, is a hallmark of modern drug discovery and can expedite the development of novel therapeutics.

The collaboration between the phytochemical examination of *Tephrosia purpurea* and the molecular docking analysis of beta-sitosterol with OmpA offers a comprehensive viewpoint on the capacity of natural products to address bacterial infections.(16) The recognized phytochemicals in *Tephrosia purpurea*, acknowledged for their anti-inflammatory, antimicrobial, and antioxidant characteristics, play a role in the traditional applications of the plant. Subsequently, the docking analysis introduces a precise molecular target, OmpA, offering a mechanistic comprehension of how beta-sitosterol could potentially exert its antimicrobial effects against *E.coli.(17)*

Nevertheless, it is crucial to recognize the constraints associated with these analyses. In silico docking investigations offer forecasts derived from computational models, and although they provide valuable insights, experimental confirmation is essential to affirm the observed inhibitory effects in practice.(17,18)Additionally, the complexities of the biological system, including potential off-target effects and the dynamic nature of protein interactions, necessitate further investigation beyond the computational predictions.

In summary, the combination of phytochemical analysis and molecular docking studies highlights the capacity of natural products to combat bacterial infections.(19)Tephrosia purpurea's diverse bioactive compounds, combined with the promising interaction between beta-sitosterol and OmpA, provide a foundation for future research.(20) This comprehensive approach aligns with the evolving trend of investigating compounds derived from nature as potential reservoirs for new therapeutics, presenting promising prospects for inventive strategies in the fight against bacterial infections.(21)

CONCLUSION:

In conclusion, our investigation revealed the presence of various phytocompounds in the plant extract through phytochemical analysis. Notably, the in silico analysis highlighted beta-sitosterol as exhibiting a robust interaction with the outer membrane protein OmpA of *E.Coli*. This suggests a potential inhibitory effect on the virulence mechanism of *E. coli*, specifically targeting a key protein involved in bacterial pathogenicity. The cumulative findings indicate that the phytocompounds identified in the plant extract, particularly beta-sitosterol, hold promise for antimicrobial activity. While further experimental validation is essential, this integrated approach aligns with the contemporary exploration of natural compounds as potential sources for novel

antimicrobial agents, opening avenues for future research in the development of alternative strategies against bacterial infections.

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