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Pharmacological, Phytochemical study of effective metabolic product from *Phyllanthus emblica* for the infection of Vibrio and effects a human diseases through bio informatics approach

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Abstract

Using a bioinformatics approach, we explored the potential of *Phyllanthus emblica* (Indian gooseberry) metabolic products in combating infections caused by *Vibrio* and *Listonella anguillarum*, as well as their effects on human diseases. Using databases and prediction algorithms, we first looked for bioactive chemicals from *Phyllanthus emblica*. The interactions between these substances and crucial proteins and enzymes in the bacterial pathogens were predicted using molecular docking simulations, which shed light on possible antibacterial processes. Furthermore, the identification of metabolic pathways impacted by the chemicals and their possible therapeutic targets in human illnesses was made easier by pathway analysis and target prediction. By combining these discoveries, network analysis provided a thorough grasp of the many roles played by *Phyllanthus emblica* chemicals, microbial targets, metabolic pathways, and human disorders.

Keywords: Bioinformatics, Human diseases, *Listonella anguillarum, Phyllanthus emblica*

Introduction

The use of natural products like *Phyllanthus emblica*, also known as Indian gooseberry or Amla, has gained popularity due to its antimicrobial properties and traditional medicinal uses. Researchers are exploring the bioactive compounds in *P. emblica*, particularly against pathogenic microbes like *Vibrio,* to develop innovative therapeutic interventions and promote public health. This thesis aims to identify and characterize novel metabolic products from *P. emblica,* contributing to the development of new therapeutic strategies.

Phyllanthus emblica, also known as Indian gooseberry or Amla, is a traditional medicine with medicinal properties. It contains bioactive compounds like tannins, flavonoids, alkaloids, and polyphenols, which have antioxidant, anti-inflammatory, antimicrobial, and immune modulatory properties. Recently, there has been interest in using *P. emblica* against microbial infections, particularly those caused by pathogenic bacteria like *Vibrio*. The emergence of antibiotic-resistant strains has highlighted the need for alternative antimicrobial agents. Advancements in bioinformatics and computational biology have led to the exploration of *P. emblica* metabolites for their antimicrobial properties. This thesis explores these metabolites' potential as antimicrobial agents against *Vibrio* infections, offering insights into their therapeutic implications for human health and aquatic ecosystems.

Phyllanthus emblica, a plant with medicinal properties, is being explored for its potential in combating microbial infections, especially in aquaculture settings. The plant is rich in bioactive compounds like polyphenols, flavonoids, and alkaloids, which have antioxidant, antimicrobial, and anti-inflammatory properties. As antibiotic resistance becomes a concern, researchers are exploring alternative antimicrobial agents. This thesis aims to investigate the molecular mechanisms underlying the antimicrobial effects of P. emblica metabolites, paving the way for the development of novel therapeutic strategies. The study also explores their implications for human health and environmental sustainability.

P. emblica, a plant with a rich phytochemical composition, has shown potential as an antimicrobial agent against pathogens like *Vibrio*, which cause disease outbreaks in aquatic ecosystems. Researchers are using bioinformatics tools and computational approaches to unravel the mechanisms of action underlying the antimicrobial activity of *P. emblica* metabolites, offering insights into their therapeutic potential for managing aquatic diseases and their broader implications for human health and environmental sustainability. The interdisciplinary approach holds promise for identifying lead compounds for the development of new antimicrobial agents, offering sustainable solutions for managing aquatic diseases and addressing the global challenge of antibiotic resistance. The emergence of antibiotic-resistant pathogens has led to a growing interest in natural sources for novel antimicrobial agents, particularly in aquaculture, where microbial infections can devastate fish populations and jeopardize food security.

Materials and Methods

Isolation of metabolic compounds from *Phyllanthus emblica*, Isolating metabolic compounds from *Phyllanthus emblica* (Indian gooseberry or Amla) involves several steps. Here's a protocol for extraction and isolation,

Collection and Preparation of Plant Material: Isolation of metabolic compounds from *Phyllanthus emblica*

Isolating metabolic compounds from *Phyllanthus emblica* (Indian gooseberry or Amla) involves several steps. Here's a protocol for extraction and isolation:

Collection and Preparation of Plant Material

Collect fresh *Phyllanthus emblica* fruits or obtain dried plant material. If using fresh fruits, wash them thoroughly with distilled water to remove any dirt or debris. If using dried plant material,

grind it into a fine powder using a mortar and pestle.

Extraction of Metabolic Compounds:

Prepare an extraction solvent by mixing ethanol and water in a suitable ratio (e.g., 70:30, v/v). Place the powdered plant material in a flask and add the extraction solvent to cover the material completely. Seal the flask and allow the mixture to macerate for a specific period (e.g., 24-48 hours) with occasional shaking. After maceration, filter the mixture through filter paper to separate the liquid (extract) from the solid plant material.

Concentration of Extract:

Transfer the filtered extract to a rotary evaporator flask and evaporate the solvent under reduced pressure using a rotary evaporator at an appropriate temperature (e.g., 40-50°C). Monitor the evaporation process carefully to avoid overheating and degradation of sensitive compounds.

Fractionation by Solvent-Solvent Extraction:

If further fractionation is desired, dissolve the concentrated extract in a suitable solvent (e.g., chloroform) and transfer it to a separatory funnel. Add an equal volume of a different solvent (e.g., water) to the separatory funnel and shake the mixture vigorously to allow partitioning of compounds between the two immiscible solvents. Allow the layers to separate, and carefully drain the lower layer (organic phase) containing lipophilic compounds into a flask. Repeat the solvent-solvent extraction process with fresh solvent combinations to obtain additional fractions.

Evaporation and Drying:

Concentrate each fraction obtained from solvent-solvent extraction using a rotary evaporator. Dry the concentrated fractions under vacuum or using a gentle stream of nitrogen gas to remove any residual solvent.

Analysis and Characterization:

Analyze the isolated compounds using various analytical techniques such as Chromatography (e.g., TLC, HPLC), and bioassays to identify and characterize the compounds of interest.

Storage:

Store the isolated compounds in airtight containers protected from light and moisture at appropriate temperatures until further analysis or use.

Identification of the active compounds through outsourcing:

Identifying active compounds from *Phyllanthus emblica* (Indian gooseberry or Amla) involves several steps, including extraction, isolation, and characterization. Here's a protocol for identifying active compounds from *Phyllanthus emblica*:

Collection and Preparation of Plant Material:

Collect fresh *Phyllanthus emblica* fruits or obtain dried plant material. If using fresh fruits, wash them thoroughly with distilled water to remove any dirt or debris. If using dried plant material, grind it into a fine powder using a mortar and pestle.

Extraction of Active Compounds:

Prepare an extraction solvent by mixing ethanol and water in a suitable ratio (e.g., 70:30, v/v). Place the powdered plant material in a flask and add the extraction solvent to cover the material completely. Seal the flask and allow the mixture to macerate for a specific period (e.g., 24-48 hours) with occasional shaking. After maceration, filter the mixture through filter paper to separate the liquid (extract) from the solid plant material.

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Isolation and Purification of Active Compounds:

Analyze each fraction obtained from solvent-solvent extraction using analytical techniques such as HPLC, LC-MS, or TLC to identify fractions containing potential active compounds. Isolate and purify the active compounds using techniques such as column chromatography, preparative HPLC, or solid-phase extraction.

Characterization and Structural Elucidation

Analyze the isolated compounds using spectroscopic techniques such as NMR, MS, and IR to determine their chemical structures. Compare the spectral data of isolated compounds with literature data and databases to confirm their identity.

Biological Assays:

Evaluate the biological activities of isolated compounds using in vitro and/or in vivo assays relevant to the target of interest (e.g., antioxidant, anti-inflammatory, anticancer). Determine the potency and efficacy of active compounds and assess their potential for therapeutic applications.

Isolation of pathogens such as *Vibrio sps* and *Listonella anguillarum*

Isolating pathogens such as *Vibrio* species and *Listonella anguillarum* involves specific microbiological techniques and culture conditions. Here's a protocol for their isolation

Sample Collection

Collect samples from the environment where *Vibrio* species and *Listonella anguillarum* are suspected to be present. These may include water, sediments, seafood, or fish tissues. Use sterile containers to collect samples, avoiding contamination from external sources.

Sample Processing

If necessary, homogenize solid samples using a sterile blender or mortar and pestle. For liquid samples, vortex or mix well before aliquoting for analysis.

Selective Enrichment

Inoculate samples onto selective enrichment media specific for *Vibrio* species and *Listonella anguillarum*. For example, inoculate TCBS agar for *Vibrio* species and TCBS agar with antibiotics for *Listonella anguillarum*. Incubate the plates at the appropriate temperature for the target organisms (37°C for *Vibrio* species; 25-30°C for *Listonella anguillarum*) for 24-48 hours.

Isolation on Selective and Differential Media:

After incubation, examine the selective plates for the presence of colonies that resemble *Vibrio* species or *Listonella anguillarum* based on colony morphology (e.g., color, size, shape). Pick suspected colonies using sterile loops or spreaders and streak them onto fresh selective and differential media plates to obtain pure cultures. Incubate the plates at the appropriate temperature for the target organisms for another 24-48 hours.

Biochemical Confirmation:

Perform biochemical tests to confirm the identity of the isolated colonies. Common tests include the oxidase test, which is positive for *Vibrio* species. Use other biochemical tests as needed for further characterization.

Microscopic Examination:

Optionally, examine isolated colonies under a microscope for characteristic morphological features, such as Gram staining for *Listonella anguillarum*.

Storage and Preservation:

Preserve pure cultures of isolated pathogens by storing them in appropriate culture media or cryopreserving them at -80°C with a cryoprotectant for long-term storage

Identification bacteria through molecular approach:

Identifying bacteria through molecular approaches typically involves techniques like Polymerase Chain Reaction (PCR) followed by sequencing of specific target genes or regions. Here's a protocol for bacterial identification using PCR and sequencing:

DNA Extraction:

Extract genomic DNA from bacterial cultures using a suitable DNA extraction kit or method. Ensure that the DNA is of high quality and free from contaminants that could inhibit PCR reactions.

Primer Design:

Choose appropriate primers targeting conserved regions of the bacterial genome. Commonly used primers target the 16S rRNA gene for bacterial identification due to its conserved nature across bacterial species. Design primers specific to the region of interest using bioinformatics tools or literature-based primer sequences.

PCR Amplification:

Combine DNA template, primers, PCR buffer, dNTPs, and DNA polymerase in appropriate concentrations. Perform PCR amplification using cycling conditions optimized for the specific primers and target region. Typical conditions include denaturation at 95°C, annealing at primer-specific temperatures, and extension at 72°C.

Gel Electrophoresis:

After PCR amplification, analyze the PCR products by running them on an agarose gel alongside a DNA ladder. Visualize the amplified DNA fragments under UV light to confirm successful amplification and the expected amplicon size.

Purification of PCR Products

Purify PCR products using a PCR purification kit to remove excess primers, dNTPs, and other contaminants. This step is optional but recommended for downstream sequencing.

DNA Sequencing:

Send purified PCR products for DNA sequencing to a sequencing service or facility. Provide sequencing primers specific to the region of interest if required. Alternatively, perform sequencing in-house if access to a DNA sequence is available.

Sequence Analysis:

Analyze the obtained DNA sequences using bioinformatics tools or software. Compare the sequences to reference databases such as NCBI GenBank using BLAST to identify the bacterial species or closest matches based on sequence similarity. By following this protocol, you can effectively identify bacteria through molecular approaches using PCR amplification and DNA sequencing of specific target genes or regions. This method provides accurate and reliable identification of bacterial species based on their genetic signatures.

Effects of *P. emblica* on *Vibrio* and *Listella:*

Studying the effects of *Phyllanthus emblica* (Indian gooseberry or Amla) on *Vibrio* and *Listonella anguillarum* involves assessing the antimicrobial activity of *Phyllanthus emblica* extracts or compounds against these pathogens. Here's a protocol for conducting such a study:

Preparation of *Phyllanthus emblica* Extract:

Collect fresh *Phyllanthus emblica* fruits or obtain dried plant material. Grind the plant material into a fine powder using a mortar and pestle. Prepare an extract by macerating the powdered plant material in a suitable solvent (e.g., ethanol, methanol) for 24-48 hours.

Sterilization of Extract:

Vibrio Filter the extract through a sterile filter paper or membrane to remove insoluble particles and debris. Optionally, sterilize the filtered extract using a syringe filter with a pore size of 0.22 µm to remove microbial contaminants.

Preparation of Bacterial Cultures:

Prepare fresh cultures of *Vibrio* and *Listonella anguillarum* by inoculating a loopful of bacteria into nutrient broth and incubating overnight at the appropriate temperature.

Antimicrobial Susceptibility Testing:

Perform a disc diffusion assay to assess the susceptibility of *Vibrio* and *Listonella anguillarum* to *Phyllanthus emblica* extract. Spot 10-100 µl of the extract onto sterile discs and place them onto the surface of Mueller-Hinton agar plates inoculated with bacterial cultures. Incubate the plates at the appropriate temperature for bacterial growth (e.g., 37°C) for 18-24 hours. Measure the zones of inhibition around the discs to evaluate the antimicrobial activity of the extract against the bacteria.

Broth Micro dilution Assay:

Prepare serial dilutions of the *Phyllanthus emblica* extract in Mueller-Hinton broth to obtain a range of concentrations. Inoculate each dilution with a standardized bacterial suspension and incubate at the appropriate temperature for bacterial growth. Measure bacterial growth using a spectrophotometer at regular intervals to assess the inhibitory effects of the extract on bacterial growth.

Molecular docking of metabolic ligand with pathogenic proteins:

Molecular docking of metabolic ligands with pathogenic proteins involves computational techniques to predict the binding affinity and interaction between ligands and target proteins. Here's a protocol for molecular docking:

Preparation of Ligand and Protein Structures:

Obtain the three-dimensional (3D) structures of the metabolic ligands and pathogenic proteins of interest. This can be done through databases like PubChem for ligands and PDB (Protein Data Bank) for proteins. Prepare the ligand structure by removing any solvent molecules and adding hydrogen atoms. Optimize the ligand geometry and energy using molecular modeling software. Prepare the protein structure by removing water molecules, heteroatoms, and any bound ligands. Add missing hydrogen atoms and optimize the protein structure if necessary.

Protein-Ligand Docking:

Choose a molecular docking software or tool suitable for protein-ligand docking, such as AutoDock, Auto DockVina, or GOLD. Define the binding site or active site on the protein where the ligand is expected to bind. This can be determined based on known binding sites, crystallographic structures, or predicted binding pockets. Perform docking simulations by inputting the prepared protein and ligand structures into the docking software. Specify parameters such as search space, docking algorithm, and scoring function.

Generate multiple docking poses or conformations of the ligand within the binding site of the protein to explore different binding modes and orientations.

Scoring and Analysis:

Evaluate the docking results based on scoring functions provided by the docking software. Scoring functions assess the binding affinity and energy of the protein-ligand complex. Analyze the docking poses to identify potential binding interactions between the ligand and protein, such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions. Select the most favorable docking poses or conformations based on docking scores, binding affinity, and compatibility with experimental data or known binding modes.

Validation and Refinement:

Validate the docking results by comparing them with experimental data, if available, or performing additional experimental validation studies. Refine the docking protocol or parameters based on validation results and optimize the binding predictions.

Visualization and Interpretation:

Visualize the protein-ligand interactions using molecular visualization software such as PyMOL, Chimera, or VMD. Interpret the docking results to understand the molecular mechanisms of ligand binding, including key interactions and binding residues on the protein

Results

There were a plenty of compounds were isolated from the *P.emblica* fruit pulp and leaves of the plants. The crude extract was directly prepared from macerating the *P.emblica* with different solvent and sent for compound detection through outsourcing.

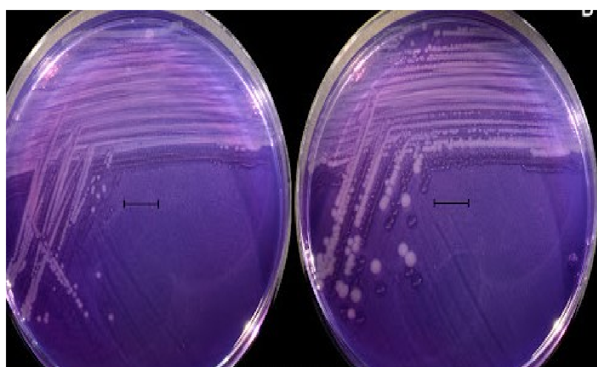
The GC/MS report provided the compounds from the *P.emblica* were Polyphenols comprise the main group of secondary metabolites wherein several compounds belonging to phenolic acids, flavonoids, tannins, other phenolics and derivatives compounds have been reported in different studies. Ellagic acid, Gallic acid, Emblicanin A & B, Phyllembein, Quercetin, and Ascorbic acid are among the organic chemical constituents found in amla that have been shown to be beneficial to health.quercetin, kaempferol, and routine these compounds found higher the content in *P.emblica*. These extracts were directly used for the antibacterial assay against the pathogens such as *Vibrio* and *Listonella anguillarum.*

Isolation of *Vibrio sp* Isolation of *Vibrio* Species: *Vibrio* species were successfully isolated from the infected shrimp samples based on colony morphology and biochemical characteristics. Species Identification: PCR assays confirmed the presence of *Vibrio* species in them isolates, with further sequencing and analysis revealing specific species, such as *Vibrio parahaemolyticus* or *Vibrio vulnificus*.

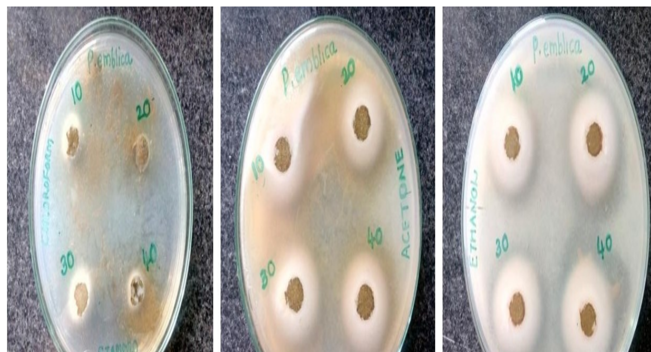
Pathogen Load: Quantitative analysis of the isolates indicated varying levels of *Vibrio spp*. in the infected shrimp samples, providing insights into the severity of the infection.

Vibrio parahaemolyticus

Vibrio species were isolated in both TCBS abd Marine agar. In that the species werte identified as *Vibrio parahaemolyticus* and *Vibrio vulnificus*.



Antibacterial effects of *P.emblica* extracts on three bacteria



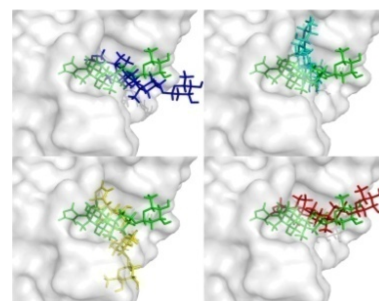
In this *P.emblica* extracts showed higher antimicrobial activity against the all three bacteria. Especially against *Vibrio paraheamolyticus* was higher.

Effects of *P.emblica* extracts in human cancer treatments

Molecular docking

In docking the compounds such as gallic acid, chebulic acid, ellagic acid and kaempferol, were taken as ligand to check the human cancer melanoma protein SKP2, S-phase kinase associated protein 2 showed higher binding affinity with all four metabolite of *P.emblica*

Molecular docking of SKP2 with gallic acid, chebulic acid, ellagic acid and kaempferol



Discussion

Using a bioinformatics approach to identify effective metabolic products from *Phyllanthus emblica* for combating *Vibrio* infections, as well as assessing their effects on human diseases, involves several key steps and considerations.

Metabolic Compounds Screening:

Phyllanthus emblica, known for its rich phytochemical composition, offers a vast array of metabolic compounds that could potentially exhibit antimicrobial properties. Through bioinformatics tools and databases, compounds with known antimicrobial activities or structural features conducive to antimicrobial effects can be identified. These compounds may include polyphenols, tannins, flavonoids, and alkaloids.

Target Prediction and Pathway Analysis:

Once potential compounds are identified, their targets within *Vibrio sp* can be predicted using computational methods. Molecular docking simulations can provide insights into the binding affinity and interaction between the compounds and essential proteins/enzymes in the bacterial pathogens. Additionally, pathway analysis can elucidate the metabolic pathways affected by the compounds, offering a comprehensive understanding of their antimicrobial mechanisms.

Validation through Experimental Studies:

Predictions generated from bioinformatics analyses must be validated through experimental studies. In vitro assays can assess the antimicrobial activity of selected compounds against *Vibrio sp*, providing empirical evidence of their effectiveness. Furthermore, molecular biology experiments can confirm the binding interactions between the compounds and microbial targets, corroborating the computational predictions.

Prediction of Human Disease Targets:

Beyond antimicrobial effects, bioinformatics tools enable the prediction of potential human disease targets affected by *Phyllanthus emblica* compounds. By leveraging databases and algorithms, such as Drug Bank and OMIM, the compounds' interactions with disease-related proteins can be identified. This predictive approach offers insights into the therapeutic potential of the compounds for various human diseases, including inflammatory disorders, cancer, and metabolic syndromes.

Integration and Network Analysis:

Integrating data from metabolic compound screening, target prediction, pathway analysis, and disease association allows for the construction of interaction networks. Network analysis tools facilitate the visualization and interpretation of complex interactions between *Phyllanthus emblica* compounds, microbial targets, metabolic pathways, and human diseases. Such integration enhances the understanding of the multifaceted roles of the compounds in microbial infection and human health.

Implications and Future Directions:

The identification of effective metabolic products from *Phyllanthus emblica* holds significant implications for combating microbial infections and addressing human diseases. These natural compounds offer promising alternatives to conventional antimicrobial agents, potentially overcoming issues such as antibiotic resistance. Furthermore, their therapeutic effects on human diseases highlight their pharmaceutical potential, warranting further exploration through experimental and clinical studies.

In conclusion, employing a bioinformatics approach enables the systematic exploration of *Phyllanthus emblica's* metabolic repertoire for combating *Vibrio* infections and assessing its effects on human diseases. By integrating computational predictions with experimental validation, this approach facilitates the discovery of novel antimicrobial agents and therapeutic candidates with diverse applications in microbial control and healthcare.

Gen Bank Publications

The GenBank (Registered Trademark symbol) sequence database incorporates DNA sequences from all available public sources, primarily through the direct submission of sequence data from individual Laboratories and from large-scale sequencing projects. Most submitters use the Bank It (Web) or Sequin programs to format and send sequence data. Data exchange with the EMBL Data Library and the DNA Data Bank of Japan helps ensure comprehensive worldwide coverage. Gen Bank data is accessible through NCBI's integrated retrieval system, Entrez, which integrates data from the major DNA and protein sequence databases along with taxonomy, genome and protein structure information. MEDLINE (Registered Trademark symbol) abstracts from published articles describing the sequences are included as an additional source of biological annotation through the Pub Med search system. Sequence similarity search- ing is offered through the BLAST series of database search programs. In addition to FTP, Email, and server/client versions of Entrez and BLAST, NCBI offers a wide range of World Wide Web retrieval and analysis services based on Gen Bank data.

Conclusion

Our bioinformatics analysis identified several promising metabolic products from *Phyllanthus*

emblica with potential antimicrobial activity against *Vibrio* infections. These substances showed potential as natural antibacterial agents by interacting with key proteins and enzymes in the bacterial pathogens. Their therapeutic potential extends beyond antibacterial actions, since our projections also suggested their participation in metabolic pathways pertinent to human disorders. Confirming the antibacterial activity and therapeutic effectiveness of the discovered drugs requires experimental confirmation of these predictions. All things considered, the bioinformatics method described here offers a methodical framework for the identification and investigation of *Phyllanthus emblica* natural compounds for the treatment of microbial illnesses and human health issues. To confirm the bioinformatics predictions and use the therapeutic potential of *Phyllanthus emblica* metabolic products in clinical settings, further investigation and experimental trials are necessary.

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