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Virtual Screening to Identify the Protein Network Interactions of Carica papaya Active Compounds with Endodontic Pathogens

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Abstract: Introduction
The Carica papaya plant possesses significant medicinal characteristics. The utilization of various components of papaya such as leaves, seeds, fruit, latex and other plant parts for medicinal purposes has been well-established and is practised on a global scale. The antibacterial properties of Carica papaya have been extensively documented. This study is to determine the interaction of phytochemical compounds of Carica papaya with endodontic pathogens using molecular docking methods.
Materials and Method
The protein interaction of 10 compounds from carica papaya was done against endodontic pathogens- Enterococcus faecalis V583, Fusobacterium nucleatum ATCC 10953 and P. micra using Stitch Software. The virulence properties of each protein interacting with the drug were tested using VirulentPred and the functional properties via VICMpred.
Results
Quercetin was found to be an active compound that strongly interacted with all three pathogens. Ferulic acid, myricetin, and caffeic acid interacted with proteins of E. faecalis V583 and F. nucleatum ATCC 10953, whereas other compounds did not interact. In P. micra, myricetin and prunasin interacted with proteins, and other compounds did not interact. Quercetin was found to interact with three crucial virulent proteins in E. faecalis and quercetin and myricetin was found to interact with two virulent proteins in P. micra.
Conclusion
The results obtained in this study suggest that bioactive compounds from Carica papaya may have potential to interact with bacterial proteins of three potential endodontic pathogens. These findings make it potentially suitable for the development of a new drug in the treatment of apical periodontitis.

Keywords: Carica papaya, quercetin, binding affinity, anti-bacterial property, E. faecalis, F. nucleatum, P. micra.

1. Introduction

The effectiveness of herbal extracts as antibacterial agents may exhibit variation depending on a number of factors including the particular extract used and the specific bacterial strain against which it is directed. Numerous herbs consist of innate compounds exhibiting antimicrobial properties and have been utilized for several centuries in traditional medicine to treat diverse infections. It is crucial to note that the effectiveness of herbal extracts against bacteria may be influenced by multiple factors, including extract concentration and quality, extraction methodology, and bacterial susceptibility.

The Caricaceae family, which has four genera worldwide. There are four species in India that belong to the genus Carica Linn, with Carica papaya Linn being the most frequently farmed and well-known species. The papaya's leaves, fruits, seeds, and latex are utilised for a variety of industrial and medical processes. Numerous phytochemicals, including vitamins, enzymes, minerals, polysaccharides, proteins, lipids, oils, lectins, sterols,

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saponins, and flavonoids, are present in papaya extract (1). The antibacterial efficacy of Carica papaya extract has been studied to evaluate its potential as a natural antimicrobial agent.

The various bioactive components of papaya extract, including papain, chymopapain, carpaine, and flavonoids, are responsible for its possible antibacterial properties. These substances have demonstrated antibacterial activity against specific microorganisms in laboratory experiments. The most effective plant ingredient for therapeutic significance is alkaloids. Because of their analgesic, antispasmodic, and antibacterial qualities, pure isolated alkaloids and their synthetic derivatives are used as fundamental therapeutic agents (2),(3,4).

Endodontic pathogens refer to microorganisms that commonly exist in the root canal system of teeth and are connected with endodontic infections. The genesis of these infections is predominantly attributed to dental caries, dental trauma, or invasive dental procedures that facilitate bacterial infiltration into the root canal. The most common endodontic pathogens include- Enterococcus faecalis, Porphyromonas gingivalis, Prevotella spp, Fusobacterium nucleatum, P.micra, Streptococcus species. The primary etiological factor of apical periodontitis in the root canal system is attributed to infection of the pulp tissue triggered by caries or other pathways. Kakehashi et al.'s landmark study revealed that the exposure of the pulps of teeth to oral microorganisms triggered the onset of pulp necrosis and peri-radicular inflammation in conventional rats. There exists substantial evidence indicating that bacteria play a substantial role as an etiologic factor in the pathogenesis of apical periodontitis. Bacterial toxins such as lipopolysaccharide (LPS) and lipoteichoic acid (LTA), along with harmful metabolic byproducts that exit from the root canal system and penetrate into the periapical tissues, possess the ability to incite a periapical immunoinflammatory response.

There is a global need to find newer antimicrobial medicines due to the resistance of bacteria to synthetic medications (5),(6,7). Recently, there has been renewed interest in the long-standing practice of using plants and the various parts of them to cure and prevent various ailments. The aim of this study is to determine protein network interactions of Carica papaya active compounds with endodontic pathogens.

2. Materials And Methods

Ten compounds from Carica papaya were selected from the literature (Table 1). This includes 2-Methoxy-4-vinylphenol, Caffeic acid, Carpaine, Equisetin, Ferulic acid, Kaempferol, Myricetin, Prunasin, Quercetin and Tenuazonic acid. These 10 compounds from carica papaya were downloaded from the PubChem database in SDF format (8). The SDF file was imported as PyRx and energy minimization of the ligands was performed and further conversion of all the ligands into AutoDock PDBQT format was done. The Stitch software was used to identify protein interactions between endodontic pathogens- Enterococcus faecalis V583, Fusobacterium nucleatum ATCC 10953 and P. micra and the active compounds of Carica papaya (Fig. 1A, B, C). Furthermore, the virulence properties of each protein interacting with the drug were tested using VirulentPred and the functional properties via VICMpred. The scores provided by these algorithms were verified based on their amino acid sequences and patterns and were divided into two groups, that is, virulent and avirulent. Subcellular localization of virulent proteins, as predicted by PSORTb V3.0.3 (Table 2). Finally, the protein epitopes were identified using BepiPred 2.0, and the topmost peptide of E. faecalis and P.micra (Fig. 2 and 3).

3. Results

The stitch software which was used to identify drug and protein interaction of endodontic pathogens revealed that Quercetin was found to be an active compound that strongly interacted with all three pathogens. Ferulic acid, myricetin, and caffeic acid interacted with proteins of E. faecalis V583 and F. nucleatum ATCC 10953, whereas other compounds did not interact (Fig. 1A, B). In P. micra, myricetin and prunasin interacted with proteins, and other compounds did not interact (Figure 1C). VirulentPred that was used to assess the virulence properties of each protein interacting with the drug showed that Quercetin was found to interact with three crucial virulent proteins in E. faecalis and quercetin and myricetin was found to interact with two virulent proteins in P. micra (Table 1).

4. Discussion

Endodontic infections are polymicrobial diseases. Bacterial virulence factors encompass both structural components and metabolites generated by bacterial activity. The latter is attributed with causing direct harm to the pulp tissue, while the structural constituents of bacterial cells, such as lipopolysaccharides (LPS) and lipoteichoic acid (LTA), are capable of inducing tissue injury indirectly through the triggering of an immune response (9).

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Enterococcus faecalis is one of the most prevalent bacteria found in failed root canal treatments. It is a facultative anaerobic bacterium that can survive and thrive in the root canal environment. E. faecalis is known for its resistance to antimicrobial agents and its ability to form biofilms, making it challenging to eradicate. Fusobacterium nucleatum is an anaerobic bacterium that is commonly found in polymicrobial infections, including endodontic infections. It plays a significant role in the establishment and progression of dental biofilms and can contribute to the virulence and persistence of the infection. Parvimonas micra, a Gram-positive anaerobic bacterium, is frequently observed in diverse regions of the human body, including the oral cavity. Parvimonas micra is a bacterium that is commonly linked to dental infections in the oral cavity, including periodontal disease, periapical abscesses, and endodontic infections (10).

Medicinal plants have been reported to have antioxidant, antibacterial, or antimicrobial activities that have been credited to the presence of secondary metabolites such as alkaloids, flavonols, flavones, tannins, saponins, steroids, and other secondary metabolites (11). Many studies have also reported that medicinal plants contain a wide variety of free radical scavenging molecules, which act against bacterial diseases.

Antioxidants are chemical compounds that can potentially provide cellular protection against the detrimental effects of reactive species like free radicals. There exist several benefits of utilizing plants and plant-derived phytoconstituents over pharmaceutical agents. The biological activities of plant extracts and their phytoconstituents have been widely researched and established, including antidiabetic, antihyperlipidemic, free-radical scavenging, and anti-inflammatory properties (12),(13–16).

Papaya root and leaf bioactive components demonstrated antibacterial action against human pathogenic microorganisms. When compared to gram-negative bacteria, these extracts were said to have a significant inhibitory effect on gram-positive bacteria.

(17). The antibacterial, anti-inflammatory, and antioxidant effects of these medicinal plants are linked to the presence of secondary metabolites like flavonoids, alkaloids, saponins, tannins, steroids, and other metabolites (18),(19,20). These metabolites are found in numerous plant components, including the leaf, seed, fruit, and latex, giving the Carica papaya its medicinal usefulness (18).

Several in vitro studies have reported the antimicrobial property of C.papaya leaf, seed and fruit extracts against S. aureus, E. coli, K. pneumoniae, B. subtilis, P. aeruginosa and S.boydii (21),(17),(22). The actual mechanism by which the compounds from the papaya extract interact with the bacterial proteins can be found using molecular docking methods.

In the present study, quercetin compounds from Carica papaya showed the maximum binding affinity to pathogenic bacterial proteins. The flavonoid quercetin, a prominent plant pigment and a flavonol with robust antioxidant properties, is principally present in a variety of plant-based foods, including onions, grapes, berries, cherries, broccoli, and citrus fruits. It is a multifaceted antioxidant that has been recognized for its ability to provide protection against tissue damage resulting from a variety of drug-induced toxicities (23,24). Similarly other Compounds such as ferulic acid, myricetin, Kaempferol and caffeic acid interacted with proteins of E. faecalis V583, F. nucleatum ATCC 10953 and Parvimonas micra. Quercetin and myricetin had an interaction with virulent bacterial proteins.

The utilization of In Silico methods constitutes an essential component within diverse drug development initiatives. The utilization of methodologies such as ligand or target-based computational screening processes is pervasive in numerous drug discovery investigations. The primary objective of the practice of molecular docking is to enhance comprehension and anticipate molecular recognition by detecting viable structural and energetic modes of binding, while simultaneously estimating their binding affinity. The present investigation employed molecular docking techniques to predict the manner in which minuscule molecules adhere to a distinct target topology. Moreover, the study aimed to ascertain the bonding attributes of ligands encompassing diverse conformational groupings and to establish the conceivable minimal binding energies. The present study provides an overall analysis of the significant activity exhibited by bioactive compounds sourced from Carica papaya against the bacterial target proteins used in the experiment.

5. Conclusion

The results obtained in this study suggest that bioactive compounds from Carica papaya have the potential to interact with certain bacterial proteins. Some of these interactions were against the virulent proteins of the

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endodontic pathogens. These findings make papaya compounds potentially suitable for the development of a new drug in the treatment of apical periodontitis. To find the effectiveness and to understand the exact mechanism of action in-vitro studies can be done further.

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Nil

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Table 1

Organism	Active Compound (Carica papaya)	Identifier	Protein name	VICMPred Functional Class	Virulent Pred	Virulent Pred Score
Enterococcus faecalis	Quercetin	EF_0062	5'-nucleotidase family protein	Virulence factor	Virulent	1.2510
	Quercetin	EF2610	ATP synthase F1, alpha subunit	Cellular Process	Virulent	0.2082
	Quercetin	atpG	ATP synthase F1, gamma subunit	Virulence factor	Virulent	0.5147
Parvimonas micra	Quercetin	PEPMIC_01234	5'-nucleotidase, C-terminal domain protein	Virulence factor	Virulent	-0.1653
	Quercetin and Myricetin	PEPMIC_01156	kinase domain protein	Cellular process	Virulent	1.1049

Table 1: Proteins of Enterococcus faecalis, Fusobacterium nucleatum, and Parvimonas micra interact with the active compounds of Carica papaya.

Table 2

Organism	Identifier	Virulent Protein	Subcellular Localisation of Protein	Score
Enterococcus faecalis	EF_0062	5'-nucleotidase family protein	Cell wall	10.00
	EF2610	ATP synthase F1, alpha subunit	Cytoplasmic	9.97
	atpG	ATP synthase F1, gamma subunit	Cytoplasmic	7.50
Parvimonas micra	PEPMIC_01234	5'-nucleotidase, C-terminal domain protein	Cell wall	8.97
	PEPMIC_01156	kinase domain protein	Cytoplasmic membrane	9.99

Table 2: Subcellular localization of virulent protein as predicted by PSORTb V3.0.3

Figure 1

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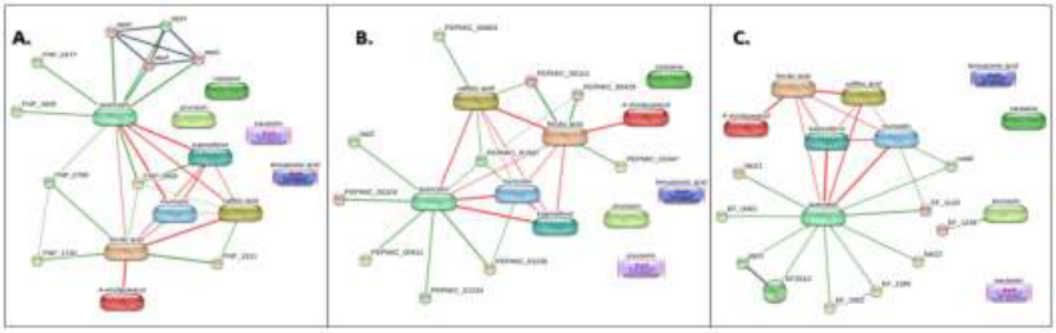

Figure 1: Protein and Drug interaction of Carcica papaya leaf active compound against Enterococcus Faecalis V583 (A), Fusobacterium Nucleatum ATCC 10953 (B) and Parvimonas micra ATCC 33270 (C). Stronger associations are represented by thicker lines. Protein-protein interactions are shown in grey, chemical-protein interactions in green and interactions between chemicals in red.

Figure 2

Figure 2: Predicted epitopes on virulent factor of Enterococcus faecalis V583 5'-nucleotidase family protein (EF_0062) (A), ATP synthase F1, alpha subunit (EF_2610) (B), and ATP synthase F1, gamma subunit (atpG) (C) identified using computational tools and the list of predicted peptide epitopes on the virulent proteins identified.

Figure 3

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Figure 3: Predicted epitopes on virulent factor of Parvimonas micra ATCC 33270 5'-nucleotidase, C-terminal domain protein (PEPMIC_01234) (A) and kinase domain protein (PEPMIC_01156) (B) identified using computational tools and the list of predicted peptide epitopes on the virulent proteins identified.

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