Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2751-2758 | ISSN:2251-6727

An in Silico Analysis to Identify Proteins Targeted by Rosmarinic Acid in Common Dental Pathogens

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(Received: 27 October 2023 Revised: 22 November Accepted: 26 December)

KEYWORDS
Rosmarinic acid
Pathogens
Interaction
Proteins
Virulence
Epitopes

ABSTRACT:

Introduction: A computational approach was used to discover the molecular targets of Rosmarinic acid (RA) in common dental pathogens. This research employed virtual simulations and data analysis to identify particular protein interactions and pathways influenced by RA. .

Objectives: The aim of this study was to provide valuable insights for developing targeted treatments against dental diseases such as caries and periodontitis.

Methods: The research utilized a computational design that employed multiple tools to detect the molecular targets of RA in five pathogens: Streptococcus mutans UA159, Enterococcus faecalis, and red complex pathogens viz., Porphyromonas gingivalis ATCC 33277, Tannerella forsythia ATCC 43037, Treponema denticola ATCC 35405. The STITCH tool was used to predict the potential protein targets of RA, while VIMCPred was used to identify the functional class of these protein targets. Additionally, BepiPred was used to predict the number of epitopes present in the virulent protein.

Results: Rosmarinic acid was discovered to interact with metabolism-related proteins of S. mutans. The serine protease HtrA was identified as the virulent protein. In E. faecalis, most of the targeted proteins belonged to the category of cellular processes. Interestingly, the virulent protein in E. faecalis was also found to be a serine protease. Multiple virulent factors have been identified in the red complex pathogens, including aminotransferase, alanyl dipeptidyl peptidase, and dipeptidyl peptidase in P. gingivalis, isoforms of trypsin domain/PDZ, hypothetical protein, and prolyl endopeptidase in T. denticola, and peptidase, S9A/B/C family, peptidase C13 family, and ATP-dependent Clp endopeptidase in T. forsythia.

Conclusions: The present study demonstrated potential molecular targets of RA in dental pathogens. The results indicated that RA can be developed as a therapeutic lead for caries and periodontal diseases as they target virulent proteins of the disease-causing pathogens. However, more intense experimental research is warranted to provide evidence of the effect of RA against important oro-dental pathogens.

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1. Introduction

Rosmarinic acid (RA) is a naturally occurring phenolic compound that is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid. RA has been found to have remarkable biological effects, including antiviral, antibacterial, anticancer, antioxidant, anti-aging, antidiabetic, cardioprotective, hepatoprotective, nephroprotective, antidepressant, antiallergic, and anti-inflammatory activities [1]. Studies have shown that RA and some compounds isolated from rosemary extracts, such as carnosic and ursolic acids and carnosol, have the ability to reduce the likelihood of tumor development in several body organs, including the stomach, colon, liver, breast, and leukemia cells. RA also has hypolipidemic effects, which can help lower lipid levels in the blood. In rats, RA is partially metabolized to coumaric acid and caffeic acid, and the hypolipidemic effect of RA may be due to the action of its metabolites [2]. Caffeic acid, a metabolite of RA, has been found to inhibit the synthesis of hepatic fatty acid synthase, 3-hydroxy-3-methylglutaryl CoA reductase, and acyl-CoA: cholesterol acyltransferase activities, and increase fatty acid β-oxidation activity in high-fat diet-induced obese mice. This suggests that caffeic acid can be used to treat obesity-related conditions [3]. Furthermore, caffeic acid and sinapic acid, another metabolite of RA, have been found to increase serum estradiol concentrations in rats with estrogen deficiency, contributing to the observed metabolic effects. The wide range of biological effects of RA and its metabolites suggest that this natural compound has significant potential for use in the treatment of various diseases and conditions.

In line with the above facts, Rosmarinic acid was assessed for its interaction with common dental pathogens such as Streptococcus mutans UA159, Enterococcus faecalis, and red complex pathogens viz., Porphyromonas gingivalis ATCC 33277, Tannerella forsythia ATCC 43037, Treponema denticola ATCC 35405. Oral diseases are a significant public health issue that affects people worldwide. According to the World Health Organization (WHO), dental caries, periodontal disease, tooth loss, and oral cancers are among the most common and impactful oral health conditions globally. Unfortunately, the prevalence and impact of oral diseases are more significant in marginalized populations, including low-income and middle-income countries. Social and economic inequalities exacerbate the situation, as people living in poverty may not have access to basic dental care, leading to untreated oral diseases that can result in severe complications [4]. Despite being mostly preventable, oral diseases persist due to inadequate funding for prevention and treatment. This is especially true in low-income and middle-income countries where resources are limited. Therapeutic strategies employing antibiotics face threats due to the emergence of drug resistance in pathogens. An alternative method suggested by researchers is the use of herbal medicine for the treatment of such problems because of less toxicity, easy availability, and cost-effectiveness [5]. The bioactive compounds present in the herbs and plants could be analyzed for their antimicrobial potential against common dental pathogens.

2. Objectives

The objective of the research is to identify proteins targeted by rosmarinic acid in common dental pathogens. This study aims to understand the mechanism of action of rosmarinic acid, which could lead to the development of new therapeutic agents against dental pathogens. The research focuses on molecular targets for potential dental treatments.

3. Methods

Strains and phytocompound used in the study

The phytocompound Rosmarinic acid was tested against dental pathogens, namely Streptococcus mutans UA159, Enterococcus faecalis, and red complex pathogens viz., Porphyromonas gingivalis ATCC 33277, Tannerella forsythia ATCC 43037, Treponema denticola ATCC 35405. STITCH tool revealed the interaction between the compound and protein repertoire in the pathogen (http://stitch.embl.de/) [6].

Analyzing protein Interaction Network

STITCH Version 5.0 is an advanced pipeline that predicts how chemicals and proteins interact with each other. This interaction can take two forms: direct or physical and indirect or functional association based on primary databases' data. To predict the virulence of proteins, the pipeline utilized a range of proteins from Streptococcus mutans, Enterococcus faecalis, and red complex pathogens known to interact with rosmarinic acid. To perform this, the sequences in FASTA format were extracted from the National Center for Biotechnology Information domain. By analyzing these

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 2751-2758 | ISSN:2251-6727

sequences, the pipeline could predict proteins' functional class and virulence properties (https://www.ncbi.nlm.nih.gov/protein/?term) [7].

Prediction of functional class of interacting protein

The VICMpred server (http://crdd.osdd.net/raghava/vicmpred/) is a powerful tool for classifying proteins into four major classes. It uses advanced algorithms to identify the specific characteristics of each protein, including its virulence factor, information and storage processing, cellular processes, and metabolism. Additionally, the server can identify a range of other protein traits, including anchorage-dependent protein effluent pumps, transporters, toxins, and hemolytic molecules. All of this is made possible through the Support Vector Machine (SVM) algorithm, which analyzes each protein's amino acid composition pattern to make accurate classifications. With its sophisticated technology and detailed analysis, the VICMpred server is an essential resource for anyone working in the field of protein research [8].

Prediction of virulence properties of interacting protein

In developing drugs or phytocompounds meant for antimicrobial purposes, it is vital to determine the bacterial proteins they target. VirulentPred (bioinfo.icgeb.res.in/virulent) is an effective method for predicting a protein's virulence. This tool employs a support vector machine (SVM) to examine sequence information associated with the protein. The resulting score assigned to each protein can help researchers gauge its likelihood of being virulent. Proteins with positive predicted values are more likely to be classified as virulent, while those with negative values are more likely to be classified as avirulent. This information can be incredibly useful in understanding the antimicrobial activity of a given compound and its potential for treating bacterial infections [9].

Prediction of B cell Epitope in the virulence proteins

Epitopes are essential for the development of vaccines and immunotherapies. They are specific sites on the surface of virulent proteins that can stimulate an immune response in the host's body. Identifying these B cell epitopes is crucial for developing effective vaccines and therapies against various diseases. Bepred is a powerful tool that uses computational algorithms to predict the peptide molecules that are likely to be part of the epitope. These predicted peptide molecules are then evaluated based on their score, and those that score above the threshold of >0.5 are predicted to be part of the epitope and are highlighted in yellow on the graph, making it easier for researchers to identify them. This predictive approach effectively identifies potential epitopes, reducing the time and cost required to develop new vaccines and therapies [10, 11].

4. Results

Rosmarinic acid was found to interact with metabolism-related proteins of S.mutans. The serine protease HtrA was found to be the virulent protein. In E.faecalis, most proteins targeted were of the category of cellular process. Interestingly, serine protease was found to be the virulent protein in E. faecalis. As with the red complex pathogens, multiple virulent factors have been identified: aminotransferase, alanyl dipeptidyl peptidase and dipeptidyl peptidase in P. gingivalis, isoforms of trypsin domain/PDZ, hypothetical protein and prolyl endopeptidase in T. denticola, peptidase, S9A/B/C family, peptidase C13 family and ATP-dependent Clp endopeptidase in T. forsythia were found to be the virulent proteins (Table 1, Figure 1).

Table 1: Proteins of common dental pathogens interacting with rosmarinic acid

Organism	**Identifier**	**Proteins which interacts with rosmarinic acid**	**VICMPred Functional Class**	**VirulentPred**	**Virulent Pred Score**
Streptococcus mutans	SMU_1312	aspartate aminotransferase	Metabolism	Avirulent	-0.798
	SMU_321	hypothetical protein	Cellular process	Avirulent	-0.007
	SMU_1672	**ATP-dependent Clp protease proteol**	**Metabolism**	**Virulent**	**0.0947**

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		ytic subunit			
	SMU_816	transaminase	Metabolism	Avirulent	-0.650
	SMU_2164	serine protease HtrA	Cellular process	Virulent	1.424
	SMU_24	aromatic amino acid aminotransferase	Metabolism	Avirulent	-1.655
	SMU_1826	aminotransferase	Metabolism	Avirulent	-0.558
Enterococcus faecalis	EF2372	aspartate aminotransferase	Metabolism	Avirulent	-1.341
	EF0771	ATP-dependent Clp protease proteolytic subunit	Cellular process	Avirulent	-0.594
	EF3027	**serine protease DO**	**Cellular process**	**Virulent**	**1.406**
	EF0891	aspartate aminotransferase	Cellular process	Avirulent	-1.319
	EF2790	small hydrophobic molecule transpor	Metabolism	Avirulent	-1.229
		ter protein			
	EF1706	aromatic amino acid aminotransferase	Cellular process	Avirulent	-1.696
	EF1037	aspartate aminotransferase	Cellular process	Avirulent	-0.058
	EF1314	aminotransferase AlaT	Virulence factor	Avirulent	-0.057
Porphyromonas gingivalis	PGN_1349	dipeptidyl anmino peptidase	Virulence factor	Avirulent	-1.069
	PGN_0756	prolyl oligopeptidase	Cellular process	Avirulent	-0.723
	PGN_1694	**alanyl dipeptidyl peptidase**	**Virulence factor**	**Virulent**	**0.377**
	PGN_1549	ATP-dependent Clp protease proteolytic subunit	Cellular process	Avirulent	-0.305
	PGN_1116	aminotransferase	Cellular process	Virulent	0.274
	PGN_0637	heat shock-related protease	Virulence factor	Avirulent	-0.276

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		htrA protein			
	PGN_0883	hypothetical protein	Virulence factor	Avirulent	-1.391
	PGN_1149	prolyl tripeptidase A	Metabolism	Avirulent	-0.828
	PGN_1469	dipeptidyl peptidase	Cellular process	Virulent	0.068
Treponema denticola	**TDE2300**	**trypsin domain/PDZ**	**Virulence factor**	**Virulent**	**1.143**
	TDE1672	ATP-dependent Clp protease proteolytic subunit	Cellular process	Avirulent	-1.251
	TDE1966	**trypsin domain/PDZ**	**Virulence factor**	**Virulent**	**0.987**
	TDE2388	ATP-dependent Clp protease proteolytic subunit	Cellular process	Avirulent	-0.749
	TDE1343	trypsin domain/PDZ	Metabolism	Virulent	0.894
	TDE0100	hypothetical protein	Metabolism	Virulent	0.132
	TDE0752	**hypothetical protein**	**Cellular process**	**Virulent**	**0.796**
	TDE1119	**prolyl endopeptidase**	**Metabolism**	**Virulent**	**0.321**
	TDE0124	rhomboid	Metabolism	Avirulent	-1.155
Tannerella forsythia	BFO_3080	peptidase	Virulence factor	Avirulent	-1.407
	BFO_1659	peptidase, S9A/B/C family	Metabolism	Avirulent	-0.056
	BFO_2851	**peptidase, S9A/B/C family**	**Cellular process**	**Virulent**	**0.235**
	BFO_3171	peptidase, M28 family	Cellular process	Avirulent	-2.086
	BFO_2675	**peptidase C13 family**	**Cellular process**	**Virulent**	**1.176**
	BFO_0848	peptidase, S54 family	Metabolism	Avirulent	-0.101
	BFO_2770	peptidase, S54 family	Metabolism	Avirulent	-1.391
	BFO_0430	peptidase Do	Virulence factor	Avirulent	-0.495
	BFO_1502	**ATP-dependent Clp endopeptidase**	**Cellular process**	**Virulent**	**0.4017**
	BFO_1419	papain family cysteine protease	Virulence factor	Avirulent	-0.830

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JCHR (2024) 14(1), 2751-2758 | ISSN:2251-6727

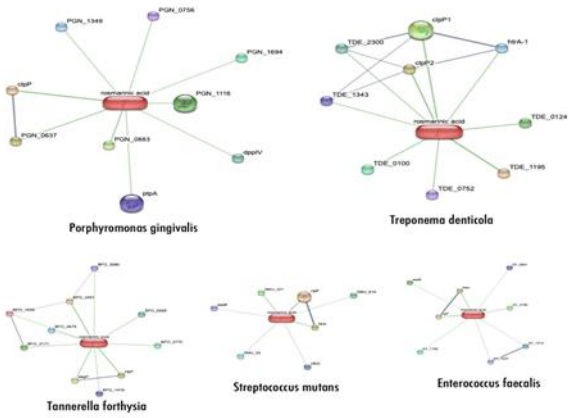

Figure 1: The protein network interactions demonstrated by oral pathogens against Rosmarinic acid

5. Discussion

Rosmarinic acid (RA) was found to interact with the caries pathogen S. mutans, E.faecalis, the pathogen that is associated with endodontic infection, and red complex pathogens that play a crucial role in the development of periodontitis. Interestingly, numerous virulent proteins of these pathogens were found to be targeted by RA. RA is an infamous phytocompound known for its antiviral, antibacterial, and anti-inflammatory effects. It acts as a defense compound and promotes health benefits when found in medicinal plants. The biosynthesis process involves enzymes from L-phenylalanine and L-tyrosine. Plant cell cultures have the potential for biotechnological production due to their ability to accumulate higher rates of the compound [12]. It has a long history of use in traditional medicine due to its medicinal properties. It is believed to have a wide range of effects such as antispasmodic, analgesic, anti-rheumatic, diuretic, and antiepileptic. The plant's secondary metabolites, including rosmarinic acid, have been extensively studied for their numerous beneficial biological activities, such as antioxidant, hepato-protective, antimicrobial, anti-nociceptive, and anti-inflammatory properties. Rosemary is also an essential ingredient in cosmetics and phytotherapy [13]. A study by Aldoghachi, aimed to evaluate the antioxidant activity and the levels of rosmarinic acid present in methanol extracts of M. piperita. High-performance liquid chromatography (HPLC) was used to determine a concentration of 1.9 mg/mL of rosmarinic acid. After purification, 0.020 g of rosmarinic acid was obtained from 1 g of crude extract. The antioxidant potential was found to be over 95% in the DPPH assay and 87.83% in the H2O2 scavenging assay.

A certain experiment employed the Oxford cup method to assess Rosmarinic acid PerillaRosA's antibacterial activity against Escherichia coli, Staphylococcus aureus, Salmonella, and Bacillus subtilis. Results indicated RosA's efficacy against all bacteria, with E. coli showing MIC and MBC at 0.8 and 0.9 mg/ml, respectively. RosA demonstrated bacteriostatic properties and inhibited bacterial cell activity [14]. A recent study demonstrated the antiviral activity of RA by employing in silico docking approaches. The binding interaction of rosmarinic acid (RA) with envelope domain III (EDIII) protein of four Dengue virus (DENV) serotypes using OCTET™ and assessed its inhibitory effect on DENV infection via plaque assay. RA demonstrated strong binding affinity and significant inhibition of all serotypes, with varying potency [15]. A study explored the biological activity of RA derivatives in the form of quaternary phosphonium salts. These derivatives exhibited significantly increased efficacy against HCT116 cells, Acanthamoeba quina, and A. lugdunensis compared to rosmarinic acid. The findings suggest potential for the synthesized compounds as promising antitumor and antiprotozoal agents [16]. A recent study by Cheng and colleagues investigated the role of rosmarinic acid in boosting the immune response of macrophages against bacterial infections. The study found that rosmarinic acid treatment increased the expression of PINK1 protein and recruited Parkin protein to mitochondria in macrophages infected with Staphylococcus aureus. This improved the macrophages' ability to fight against the bacteria, as confirmed by experiments using the Mdivi-1 drug and PINK1 gene knockdown. These findings suggest that rosmarinic acid may have the potential to enhance the immune defense mechanism against bacterial infections [17]. Methanolic extracts from three species of Salvia L. were used to analyze their phenolic contents through RP-HPLC/MS. The study found 18 detectable phenols, with kaempferol being the most abundant in S. microstegia, and rosmarinic acids being the most abundant in S. brachyantha and S. aethiopis. The antioxidant capacity was assessed using CUPRAC, FRAP, and DPPH assays, which showed moderate activity compared to standard antioxidants. The antimicrobial screening exhibited varied effectiveness.

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JCHR (2024) 14(1), 2751-2758 | ISSN:2251-6727

S. brachyantha and S. microstegia showed potent activity against certain microbes [18]. Numerous studies have been carried out using a computational approach, which offers a better understanding of the possible interactions between the phytocompound and the pathogens [19, 20].

Computational approaches have been used for a long time in drug discovery and development. These in silico methods have numerous benefits such as cost-effectiveness, reduced investigation time, real-time application of preliminary results, and many more. However, there are certain limitations to this approach. Firstly, validating the data in a biological system is necessary to confirm the findings. Secondly, the observed interactions may only be physical and have no functional consequences. Further exploration is required in such cases. Lastly, certain proteins found in bacteria may have close homology with host proteins. Therefore, in vivo experiments should be carried out to rule out any adverse side effects that may occur during the therapeutic usage of the drug.

In summary, this in silico study can potentially reveal valuable information about the molecular targets of Rosemarinic acid in dental pathogens. These findings are significant for developing targeted treatment for infection caused by dental pathogens, as they shed light on the complex molecular interactions between Rosmarinic acid and the microorganisms responsible for causing caries, endodontic, and periodontal problems. However, further research and experimental validations are necessary to fully exploit Rosmarinic acid's potential as a therapeutic agent against dental pathogens.

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