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An Observational Study on the Mode of Action of Ferulic Acid on Common Dental Pathogens – An *in Silico* Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Author JVP designed the study, performed the statistical analysis and wrote the protocol. Author RN wrote the first draft of the manuscript. Author ASSG managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In this study, the functional and virulent classes of proteins that are targeted by ferulic acid are identified from the most common dental pathogens like *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Streptococcus mutans and Enterococcus faecalis* by an *in silico* approach. The target proteins were selected using STITCH tool, the functional class of proteins was predicted using VICMpred. The predicted virulent proteins were further subjected to BepiPred analysis, which returned the number of peptide epitopes present in the protein. Further, the subcellular location of the proteins was confirmed by the PSORTb tool. Ferulic acid was found to

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interact with virulence factors such as dihydrolipoyl dehydrogenase of *Streptococcus mutans,* putative lipoprotein of *P. gingivalis* and phosphogluconate dehydrogenase of *T. forsythia.* The findings of the study emphasize the promising interactive role of ferulic acid and their potential protein targets in common oro-dental pathogens which requires further experimental validation *in-vivo* and *in-vitro*.

Keywords: Ferulic acid; STITCH tool; virulence; peptide epitopes.

1. INTRODUCTION

Targeting the oro-dental pathogens with considerable natural resources has spurred renewed interest in recent years due to the emergence of drug resistant strains that impedes dental treatment strategies. Plants encompass a diverse array of secondary metabolites with varying modes of action to efficiently target these oro-dental resistant strains. Complications in dental diseases arise mainly due to *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Streptococcus mutans and Enterococcus faecalis* [1] due to their potent virulent proteins and their mechanism of actions in the gingival mucosal layers. Phytochemicals that could efficiently interact with these virulent proteins at a cellular and molecular level would be a boon to curb the dental complications. Ferulic acid is one such bioactive phytochemical with a known potent role in interacting at a molecular level. In an organic form, it is a hydroxycinnamic acid and a phenolic chemical abundantly found in plant cell walls. Chemically, it is a component of lignin with covalently bonded side chains attached to molecules such as arabinoxylans and acts as a precursor in the manufacture of aromatic compounds [2,3]. As building blocks of lignocelluloses, ferulic acid is ubiquitously located in vegetables sources, [4] cereals such as wheat [5], flax seeds [6] and beverages [7]. In addition, it is a major metabolite of chlorogenic acids and is easily absorbed in the small intestine of humans while other forms viz., dihydroferulic acid, feruloyl glycine and dihydroferulic acid sulfate are metabolised in the large intestine by the action of gut flora [8,9]. Studies also document ferulic acid as an effective anti-oxidative [10], anti-tumour, [11] less toxic with high bioavailability [12].

Although only a few literature is available related to the mode of action of ferulic acid against oral pathogens [13], more studies are required to justify the antimicrobial action of this compound against potential dental pathogens. Interactions of ferulic acid with microbial cells result in the disruption of the cell membrane integrity, hyperpolarization of the cell membrane with reduction in intracellular pH of the microbial cell [14]. Further, ferulic acid is known to alter the intracellular potassium release, alternating the surface charges, thereby the physico-chemical surface properties of the microbial cells. Assessment of similar actions and other modes of interactions to target the virulent proteins of *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Streptococcus mutans and Enterococcus faecalis* would be a context of interest. The functional class of the virulent and interacting proteins in these pathogens with further assessment of the epitopes and their subcellular locations through computational observations will provide preliminary clues on the molecular targets. Numerous studies have been carried out using different phytocompounds against dental pathogens [15,16]. The present investigation is thus aimed to observe the protein drug interactions at a molecular level to emphasize the functional class of the interactive virulent proteins using in-silico tools and databases.

2. MATERIALS AND METHODS

2.1 Strains under Study

Using the STITCH tool, strains of *Streptococcus mutans* (UA159), *Enterococcus faecalis* (V583), *Porphyromonas gingivalis* (ATCC 33277), *Treponema denticola* (ATCC 35405), and *Tannerella forsythia* (ATCC 43037) were selected for the study [17].

2.2 Analysis of the Protein Interaction Network

STITCH tool based retrieval of the virulent proteins from the protein repertoire for predicting the direct/physical and indirect/functional interaction of the retrieved proteins from the *Streptococcus mutans, Enterococcus faecalis, Porphyromonas gingivalis, Treponema denticola* and *Tannerella forsythia* was performed. The FASTA format of protein sequences were retrieved from the National Centre for

Biotechnology Information (NCBI) domain and used for predicting the functional class of proteins and their virulence properties (https://www.ncbi.nlm.nih.gov/protein/?term).

2.3 Prediction of Functional Class of Interacting Proteins

VICMpred server aids in the classification of pathogenic microbial proteins into four major classes namely, virulence factors, information and storage processing, cellular process and metabolism. The principle virulence factors such as adhesins, toxins and haemolytic molecules are identified based on the support vector machine (SVM) algorithm which classifies proteins based on their amino acid composition and sequence pattern [18].

2.4 Prediction of B-Cell Epitopes in the Virulence Proteins

Epitopes are small regions on the antigens recognized by antibodies. Identification of B-cell epitopes on the virulence proteins identified could add more advantage to the phytocompound selected for the study. BepiPred 2.0 server was used to identify the peptide epitopes on the virulent proteins. The peptide molecules which scored above a threshold greater than 0.5 are predicted to be part of the epitope and is coloured yellow in the graph [19,20].

2.5 Prediction of Subcellular Localisation of Proteins

The identification of the subcellular localisation of virulence proteins are of prime importance as the efficiency of the compound lies in target identification. Cell surface proteins are readily targeted, whilst, the cytoplasmic or nuclear proteins need proper drug delivery systems to target the protein of interest. Hence, PSORTb was used for identification of subcellular location of virulence proteins [21].

3. RESULTS AND DISCUSSION

Ferulic acid showed a promising interaction with crucial proteins involved in cellular processes, metabolism and virulence (Table 1 and Fig. 1). A total of 39 interactions were observed between the retrieved proteins from dental pathogens. Although the compound targets functional proteins in different organisms, it targeted only the metabolism class of proteins in *T. denticola.* Additionally, dihydrolipoyl dehydrogenase of *S. mutans*, phosphogluconate dehydrogenase of *T. forsythia* and putative lipoprotein of *P. gingivalis* were found to be the virulence factors. The subcellular location of thioesterase can only be ascertained while the location for other proteins remained unknown (Table 2). Several putative epitopes were identified on the virulent proteins from different oral pathogens (Fig. 2).

The present investigation documents the computational evidence of the ferulic acid interactions with the retrieved protein from the commonest dental pathogens in addition to the assessment of its cellular locations and the putative B-cell epitope predictions. Inhibitory role of ferulic acids against microbes has already been documented in many studies [14,22,23]. Elucidation of the possible interactions of ferulic acid with the virulent protein of the dental pathogens was carried out successfully in the present study. We used STITCH tool for the prediction of the virulent proteins, which is an exhaustive tool for the identification of the physical and functional interactions between the retrieved proteins with ferulic acids.

For the functional classification of the retrieved virulent proteins, we employed a VICMpred server that aided us in the categorization of the proteins that has a role in cellular processing and metabolism, virulence and information storage mechanisms. Most of the proteins had the cellular processing function. Amidst many proteins of interaction, ferulic acid promisingly interacted with *folA* hypothetical protein that is responsible for trimethoprim resistance from the strains of *E. faecalis* and *T. forsythia*. Ferulic acid targeting *folA* is an indication for its role as a novel candidate to treat infections caused by drug resistant strains of *E. faecalis* and *T. forsythia*.

Similarly, ferulic acid showed promising interactions with four different variants of proteins viz., *bkdD, lpdA, adhD, phdD* and *PGN_0826* dihydrolipoyl dehydrogenase, the commonest being the *lpdA*. Dihydrolipoyl dehydrogenase is a flavoenzyme constituting E3 component or L-protein of five characterized 2-oxoacid dehydrogenase complex, with anticipated functions like stimulating ATP-binding cassette transport of carbohydrates [24], ubiquinone mediated amino-acid transportation [25], cell cycle progression in yeast [11] and role as an

immunogenic surface antigen [26]. These reports substantiate the role of ferulic acid in arresting the various metabolic pathways effectively in gram positive and gram negative bacteria.

Table 1. List of proteins from the common dental pathogens which interacts with ferulic acid

Organism	Identifier	Proteins which interacts with ferulic acid	VICMPred Functional Class
Enterococcus faecalis	EF_2792	Hypothetical protein	Cellular Process
	EF_1536	Hypothetical protein EF_1536	Metabolism
	EF_0294	Hypothetical protein EF_0294	Metabolism
	EF_1505	Hypothetical protein EF_1505	Metabolism
	bkdD	Dihydrolipoyl dehydrogenase	Cellular Process
	katA	Catalase	Cellular Process
	folA	Trimethoprim-resistant dihydrofolate reductase DfrE	Cellular Process
	lpdA	Dihydrolipoyl dehydrogenase	Cellular Process
	gnd	Decarboxylating 6-phosphogluconate dehydrogenase	Metabolism
	zwf	Glucose-6-phosphate dehydrogenase	Cellular Process
Streptococcus mutans	SMU_140	Glutathione reductase	Metabolism
	SMU_633	Thioesterase	Cellular Process
	SMU_1806	Glycosyltransferase	Cellular Process
	SMU_1678	Acyl-CoA thioesterase	Metabolism
	dfrA	Dihydrofolate reductase	Cellular Process
	adhD	dihydrolipoyl dehydrogenase	Virulence factor
	gor	Glutathione reductase (GR)	Metabolism
	livK	1-branched-chain amino acid ABC transporter substrate-binding protein	Cellular Process
	pdhD	Dihydrolipoyl dehydrogenase	Metabolism
	cysK	Cysteine synthase A	Metabolism
Porphyromonas gingivalis	PGN_1328	Hypothetical protein PGN_1328	Metabolism
	PGN_2061	Probable dihydrofolate reductase	Cellular Process
	PGN_1878	Putative lipoprotein	Virulence factor
	PGN_0826	Dihydrolipoamide dehydrogenase	Cellular Process
Treponema denticola	TDE_2620	Conserved hypothetical protein	Metabolism
	TDE_0192	Conserved hypothetical protein	Metabolism
	TDE_2361	High affinity branched chain amino acid ABC transporter, amino acid-binding protein	Metabolism
	TDE_0877	Conserved hypothetical protein	Metabolism
	cinI	Cinnamoyl ester hydrolase	Metabolism
	lpdA	Dihydrolipoamide dehydrogenase	Metabolism
Tannerella forsythia	BFO_1975	Hypothetical protein BFO_1975	Metabolism
	BFO_1405	Glycosyltransferase	Cellular Process
	BFO_0595	Hypothetical protein BFO_0595	Metabolism
	BFO_1189	Hydrolase, alpha/beta domain protein	Cellular Process
	lpdA	Dihydrolipoyl dehydrogenase	Cellular Process
	folA	Dihydrofolate reductase	Metabolism
	cysK	Cysteine synthase	Metabolism
	gnd	Phosphogluconate dehydrogenase	Virulence factor
	zwf	Glucose-6-phosphate dehydrogenases	Cellular Process

Table 2. Sub-cellular localisation of virulent proteins targeted by ferulic acid

Organism	Virulent protein	Subcellular localisation of protein
Streptococcus mutans	Dihydrolipoyl dehydrogenase	Cytoplasm
Porphyromonas gingivalis	Putative lipoprotein	Unknown
Tannerella forsythia	Phosphogluconate dehydrogenase	Cytoplasm

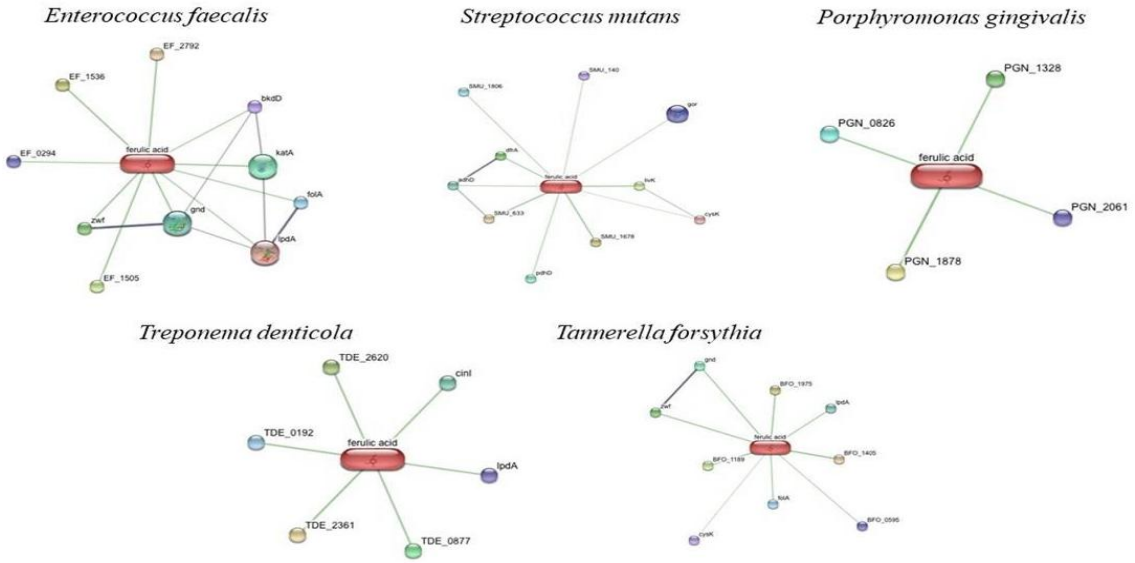

Fig. 1. Interaction of ferulic acid with the protein repertoire of common dental pathogens

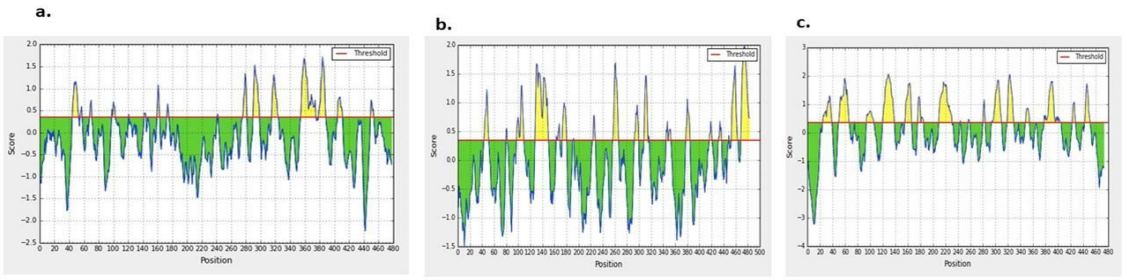

Fig. 2. Epitopes identified on the virulence factors (a) dihydrolipoyl dehydrogenase of *Streptococcus mutans* (b) putative lipoprotein of *P. gingivalis and (c)* Phosphogluconate dehydrogenase of *T. forsythia*

The present investigation also documents the interaction of ferulic acid with *zwf* hypothetical protein glucose-6-phosphate dehydrogenase, that plays a vital role in protecting the pathogens from metal induced oxidative stress [27]. It has its potent role in virulence together in exhibiting resistance to reactive oxygen and nitrogen intermediates. Interaction of ferulic acid with *zwf* protein of *E. faecalis* and *T. forsythia* indicates its candidature for novel drugs in arresting the protective role encountered by the same amidst the pathogens. As per the observation of the present study, ferulic acid may also play an enhancing role of the immune system in any host against bacteria as it shows a good interaction with glutathione reductase *(Gsr)*, *SMU_140* and *gor* proteins from *S. mutans*. *Gsr* has a role in catalyzing the reduction of glutathione disulfide to glutathione which is a major cellular antioxidant and its essential role in enhancing the host

defense against gram negative bacteria has been demonstrated in a mouse model [28]. *Gsr* with a role in sustaining the oxidative burst and enhancing the neutrophil phagocytic function [29], it can be substantiated whether ferulic acid interaction could be more beneficial in enhancing the host defense, with further *in-vivo* and *in-vitro* experimental analysis.

Interaction of ferulic acid with thioesterase *(SMU-633)* and acyl Co-A thioesterase *(SMU-1678)* in *S. mutans* was observed in the present study along with other interactions. Thioesterases and acyl coA thioesterases are considered as an enzymatic group catalyzing the hydrolysis of acyl CoA's to free fatty acids and coenzyme A. Thioesterases are involved in the metabolic regulation of peroxisome proliferation, membrane synthesis, signal transduction and gene regulation. Thus, ferulic acid can effectively inhibit the multiplication of *S. mutans* by arresting these metabolic functions in the oro-dental cavities. Ferulic acid also showed a functional interaction with cysteine synthase *(cysK)* of *S. mutans* and *T. forsythia*. Cysteine is known for its vital catalytic functions as an essential amino acid and is essential for the formation of ubiquitous proteins with iron-sulfur clusters viz., cytochromes and aconitase [30], for maintaining and intracellular reducing environment with oxidative stress protection, activation of bacterial transcriptional regulators and molecular chaperones and also for maintaining the protein folds and scaffolding in extra-cytoplasmic compartments. We observe the dihydrolipoyl dehydrogenase *adhD* from *S. mutans* to be a virulent factor. In *P. gingivalis* the putative protein *PGN 1878* with unknown localization was predicted as virulent. The hypothetical protein phosphogluconate dehydrogenase *gnd* was predicted as virulent from *T. forsythia*, in addition to its role enhancing the cell growth through the pentose pathway in bacterial cells [23]. The present investigation has successfully assessed the interaction of ferulic acid with these three virulent proteins, with a potent fact of its potent antibacterial role in targeting these dental pathogens.

4. CONCLUSION

With the concrete evidence between the interaction of the ferulic acid with the virulent and functional proteins from the commonest dental pathogens, the present study is the first of its kind to report the preliminary data of the drug-protein interactions using computational tools. However, the interactions may vary during its functional in-vivo interaction in a complex biological environment. Thus the study concludes by emphasising the need for further experimental validation both *in-vivo* and *in-vitro*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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