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PREVENTION OF *Porphyromonas gingivalis* CAUSING GUM DISEASE BY *Salvia rosmarinus*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Porphyromonas gingivalis is known as the causal organism of gum disease. One of the key enzymes involved in its biochemical pathway is gingipain R. The Biovia Discovery Studio is used to study about the molecular docking of phytochemicals with the enzyme. The strength of the interaction was evaluated based on -CDOCKER energy and -CDOCKER interaction energy. High positive values for both the parameters indicated that out of different phytochemicals, alcohol can effectively deactivate the gingipain R enzyme thereby interrupting the life cycle of *Porphyromonas gingivalis*.

Keywords: Phytochemical; *Salvia rosmarinus*; *Porphyromonas gingivalis*.

1. INTRODUCTION

Many plants are always key source of treatment strategy in different "traditional medicinal systems". In recent years, many people are choosing to plant based medicines or products to improve their health conditions or as a curative substance either alone or in combination with others. According to the WHO, herbs or herbal products are used by the large number of populations for basic healthcare needs. Herbal medicine includes herbs, herbal materials (like plant parts) or preparations, processed and finished herbal products, active ingredients [1,2].

In recent years, a huge resurgence of the use of herbal products due to the side effects of modern drugs , failure of modern therapies for afainst chronic diseases, and microbial resistance. It is estimated that nearly 75% of the plant based therapeutic entities used worldwide were included from traditional/folk medicine. In India, approximately 70% of modern drugs are discovered from natural resources and number of other synthetic analogues have been prepared from prototype compounds isolated from plants [3,4,5].

In the 21st century, pollution, unhealthy lifestyle, environmental toxins increase the risk of diseases. The side effects, overuse/misuse of drugs are also a major concern. In 2013, WHO developed and launched "WHO Traditional Medicine Strategy 2014-2023" and emphasised to integrate traditional and complementary medicine to promote universal healthcare and to ensure the quality, safety and effectiveness of such medicine [6]. Therefore the scientist are looking forward to find more plant based

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traditional medicines that would be cost effective, easily available and capable enough to provide the basic healthcare to people mostly in underdeveloped areas.

Since last few decades, there is a growing interest in traditional medicine in all over the globe. Variety, flexibility, easy availability, religious/social acceptance, relative low side effect and cost became the key factor for the growth of traditional medicine [7].

Plants provide not only essential nutrients needed for life, but also other "bioactive phytochemicals" that contribute to health promotion and disease prevention. While the macro and micronutrients in plants were long thought to be one of the essential components for human health, phytochemicals have recently emerged as modulators of key cellular signalling pathways [8,9]. "Phytochemicals", often called secondary metabolites, are non-nutritive chemical compounds produced by plants via several chemical pathways. Recent studies have demonstrated that a large number of phytochemicals can be beneficial to the function of human cells [10,11,12].

With several studies indicating the effects of phytochemical-rich foods on health, it is strongly suggested that ingesting these phytochemicals can help to improve health [13,14,15]. Based on such evidence, many researchers have previously conducted studies ton investigate the roles of phytochemicals in health improvements.

Dietary phytochemicals are commonly found in plant based foods such as fruits, vegetables, grains and tea [16]. Many studies, including animal models, population observations and clinical trials, have been conducted to investigate the protective effects of dietary phytochemical intakes from food [17-22].

Salvia rosmarinus is a medicinal plant belongs to the Lamiaceae family and is commonly called as rosemary [23]. Rosemary is a dense bush, branched, evergreen and blue white flower, reaching a height of about 1 m and have very characteristics smell [24].

In order to obtain "biologically active compounds" from rosemary, it is necessary to obtain the plant's extract and/or essential oils and perform "phytochemical characterization". The extraction methods are applied to the plant most active portions (leaves, stems, roots, or flowers), using selective solvents and standard procedures [25]. Regarding the extracts , the "phytochemicals" mainly present in rosemary are rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol [26,27,28]. Some experimental studies have reported the anti-inflammatory and analgesic activities of the essential oil and biologically active terpenes such as carnosic acid, ursolic acid and betulinic acid, as well as rosmarinic acid, rosmanol and oleanolic acid [29]. Hence plant extracts of rosemary plant are used in this study to determine its therapeutic uses against gum diseases.The presence of Porphyromonas gingivalis acting either alone or as amixed infection with other oral pathogens and possibly the deficiency of certain immunological factors in the host appears to be essential for the etiology of advanced periodontitis [30]. They are rod shaped, non motile, Gram-negative and anaerobic bacteria (NCBI). *Porphyromonas gingivalis* is the one of the most pathogenic bacteria typically causing gum diseases or periodontal diseases. This infection is a common bacterial disease that affects the oral cavity and begins as acute inflammation of the gingival tissue and untreated infections can progress to formation of teeth pockets, and eventually loss of teeth.

Porphyromonas gingivalis bacteria typically live in animals and human oral cavity and are shed through saliva. This disease technically may not be contagious however humans become infected most frequently through sharing contaminated drinking glass or utensils or even through a kiss (NCBI).

This work is therefore based on identifying the responsible phytochemicals of rosemary plant that helps in curing gum diseases caused by *Porphyromonas gingivalis.*

2. MATERIALS AND METHODS

2.1 Software Used

"Discovery studio module of Biovia software (Dassault Systemes of France) "was used for analysis. The software utilizes "machine learning techniques" to predict the level of "molecular interaction".

2.2 Methodology

2.2.1 List of phytochemicals

"Phytochemicals" are produced by plants as "secondary metabolites" to protect them from predators. The potential threats to plants include bacteria, viruses, fungi etc.. When these plants or their parts are consumed by humans these phytochemicals fight off threats to health. Some "phytochemicals" have been used as poisons and others as traditional

medicine. Published works showed that *Salvia rosmarinus* contains rosmarinic acid, tannic acid, alcohol, betulinic acid, linalool, carnosic acid etc. It has already been established that salvia rosmarinus plant belonging to Lamiaceae family has potential to help controlling gum disease. This work is focused on identification of the particular phytochemical responsible for inhibiting and controlling of gum disease.

2.2.2 Enzyme found in *Porphyromonas gingivalis*

It has been reported that gum disease can be caused as a result of *Porphyromonas gingivalis* infestation. Various metabolic cycles have been seen in the bacterial life cycle for its survival. These metabolic cycles are regulated by different enzymes. Brenda enzyme database was used to identify and list different enzymes found in *Porphyromonas gingivalis* bacteria. It has been found that gingipain R (protein database code 1CVR) is involved in Cationic antimicrobial peptides (CAMPs) resistance (KEGG). Cationic antimicrobial peptides play an important role in host defense against microbial infection and are key components of the innate immune response. CAMPs weaken the integrity of the bacterial inner and outer membranes and subsequently kill bacterial cells. On the other hand , bacteria have developed a number of mechanisms against CAMPs. These resistance mechanisms include decreased affinity to CAMPs by substituition of anionic cell surface constituents with cationic molecules; biosynthesis and crosslinking of cell envelope components ; external trapping mechanisms that bind or neutralize the CAMPs by direct secretion of proteins , or the release of CAMPs binding molecules from the host cell surface; membrane efflux pumps ; and production of peptidases (KEGG).

2.2.3 Molecular docking

"Molecular docking method" has been used to identify the phytochemical from the plant extract, that act as a ligand and form a strong covalent bond with the bacterial protein to successfully inhibit the microbe. The "Discovery studio module of Biovia software" was used for identifying "molecular interaction and perform molecular docking". In this process first the sdf files for the phytochemicals found in the *Salvia rosmarinus* plant were downloaded from the website (pubchem). The protein database code of the gingipain R enzyme was identified from the website (RCSB). The active site of the enzyme was identified via "receptor cavity" protocol found under "receptor-ligand interaction" menu. Molecular docking was done using the CDOCKER protocol of Biovia softwere under "receptor-ligand interaction". The enzyme molecule was treated as the receptor molecule and the phytochemical was treated as the ligand. The "-CDOCKER_ENERGY and -CDOCKER_INTERACTION_ENERGY" were used as indicator for the quality of "molecular docking". The high positive value of those indicators presented a good interaction between the ligand and the receptor. Thus, the interactions with high values might indicate the major phytochemical responsible for curing the disease.

3. RESULTS AND DISCUSSION

In Fig. 1 the active site of the gingipain R enzyme is shown. It appears as light green in color. CDOCK is a molecular dynamics (MD) simulated-annealing-based algorithm. It is a grid-based "molecular docking method" and optimized for accuracy. The ligand conformations were obtained by "Molecular Dynamic methods".

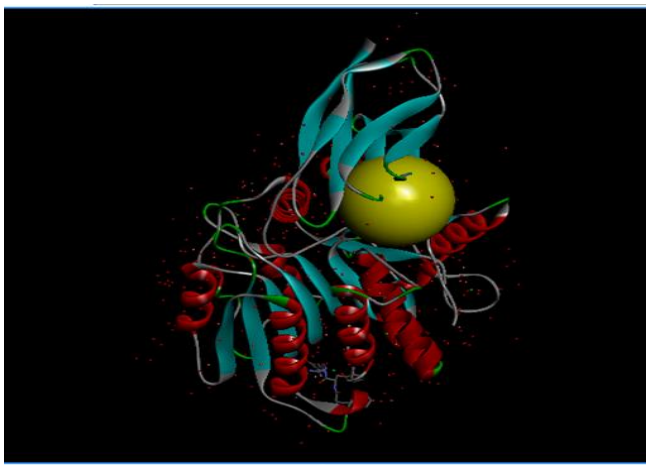

Fig. 1. Active site of gingipain R enzyme

Table 1. Results of C Docking of phytochemicals with gingipain R (receptor)

Sl. no.	Ligand	-CDOCKER energy	-CDOCKER interaction energy	Difference between -CDOCKER interaction energy and -CDOCKER energy
1	Alcohol	10.2681	11.9613	1.6932
2	Linalool	-24.8987	15.4385	40.3372
3	Rosmarinic acid	-93.5211	-24.1344	69.3867
4	Borneol	-361.586	-83.5664	278.0196
5	Tannic acid	Failed	Failed	NA
6	Betulinic acid	Failed	Failed	NA
7	Carnosic acid	Failed	Failed	NA
8	Flavones	Failed	Failed	NA

"-CDOCKER energy" was calculated based on the internal ligand strain energy and receptor-ligand interaction energy. -CDOCKER interaction signifies the energy of the nonbonded interaction that exists between the protein and the ligand. The criteria for best interaction was chosen based on (a) high positive value of -CDOCKER energy and (b)small difference between –"CDOCKER energy and -CDOCKER interaction energy" [31]. In Table 1 it is shown that gingipain R-alcohol interaction has the highest positive value of -CDOCKER energy (10.2681) and minimum value of the difference (1.6932) between - C DOCKER interaction energy and - C DOCKER energy. Thus the results indicated that alcohol as a "phytochemical" can effectively deactivate the gingipain R enzyme thereby interrupting the biological cycle of *Porphyromonas gingivalis*. Higher positive value for alcohol indicated that it was the most active ingredient against *Porphyromonas gingivalis*. On the other hand, linalool can deactivate the enzyme to a small extent (negative -CDOCKER energy but positive -CDOCKER interaction energy). Rosmarinic acid, borneol, carnosic acid, tannic acid, betulinic acid, flavones, flavonones, carnosol cannot interact with gingipain R enzyme. Thus, the key phytochemicals preventing gum disease caused by *Porphyromonas gingivalis* is alcohol.

4. CONCLUSIONS

By studying various published journals it is reported that *Salvia rosmarinus* plant has therapeutic action against gum disease (NCBI). *Porphyromonas gingivalis* is known as a major causal organism of gum disease. This study was carried out to provide the theoretical basis of this observation. Using "*Discovery* studio module of Biovia software", "molecular docking "operation was performed to identify the phytochemicals (alcohol, rosmarinic acid, carnosic acid, tannic acid, betulinic acid, linalool), which can have a significant interaction with the vital enzyme (gingipain R) of the microbe. From the above study it can be concluded that alcohol can form strong covalent bond with the enzyme successfully inhibiting the metabolic cycle of the microbe. Linalool is found to be less effective in deactivating the enzyme of the microbe. Rosmarinic acid, carnosic acid, tannic acid, betulinic acid are not effective in deactivating the enzyme. Thus, this study could explain that the presence of alcohol has been successful to enhance the medicinal values to *Salvia rosmarinus* plant against gum disease caused by *Porphyromonas gingivalis.* Since the alcohol-gingipain R interaction has been successful in inhibiting life cycle of *Porphyromonas gingivalis* by blocking its metabolic pathway like Cationic antimicrobial peptide metabolism (CAMPs) hence it is proved that phytochemical alcohol provide therapeutic values to rosemary plant against gum disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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