Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Bio Profiling of Antibacterial Activity of *Punica granatum L.*: An Ethnonutraceutical Against Cariogenic *Streptococci*

Minakshi Pandey*, R. Senthil Kumar

[1]*Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India*
[2]*School of Biotechnology, GPS Institute of Agricultural Management, Bengaluru, Karnataka, India*

ARTICLE INFO

Article history:
Received: 20 October, 2020
Revised: 15 August, 2021
Accepted: 22 August, 2021
Published: 30 September, 2021

Keywords:
Antibacterial activity,
Cariogenic bacteria,
Dental caries,
Ethno-nutraceutical,
Punica granatum L.,
Oral *streptococci*.

DOI:
10.25004/IJPSDR.2021.130501

ABSTRACT

Recently, usage of medicinal plant extracts and herbs as an alternative natural control agent for commercially existing chemical synthesized drugs in cure of oral infectious diseases like periodontal disease and dental caries has been popularized immensely. The present study was aimed to assess the efficacy of antibacterial potency of Punica granatum peel extracts on five common but major oral pathogenic bacteria such as *Streptococcus mutans, Streptococcus sanguinis, Streptococcus salivarius, Streptococcus sobrinus and Streptococcus mitis* on carcinogenesis. The anti-microbial activity was investigated using Disc infusion technique by assessing Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The extracts were prepared using water, ethanol and chloroform in different concentration by serial dilution (dilution by one-half) using sterile distilled water solvent. The crude extracts were evaluated on Brain Heart Infusion agar along with Ampicillin as positive control and water as negative control. Results obtained showed that the crude ethanolic, aqueous, chloroform extracts of *Punica granatum L.* demonstrated broad spectrum of inhibition by showing antibacterial effect against Oral streptococci bacteria. The extracts were found less effective on *S. salivarius* and more effective on *S. sanguis,* where ethanol extract showed highest effectiveness even in comparison with Ampicillin. The results exhibited in present research study leads to the scientific evidence for the primeval usage of this plant as a medicinal ethno-nutraceutical and considered to be potent and effectual alternative compared to synthetic medicines.

Introduction

The substantial discovery of antibiotics from primeval to neoteric era has beneficially helped to fight against enormous bacterial infectious diseases in humans. Virulent diseases caused by pathogenic bacteria are one of the major causes of death globally and a regular health risk factor in all countries.[1] In many bacterial strains a connate mechanism of antimicrobial resistance has been identified that severely jeopardized the usage of antibiotics. Due to indiscriminate and frequent use of antibiotics, toxic reactions have resulted in drug resistant mutants during treatment of human's chemical system. Periodically such new resistant mutants decrease the efficacy of current treatments leading to serious public health risks.[2] Healthcare providers are perturbed due to drug resistance among bacteria, as the pathogenic infection caused by them is difficult to cure. Accordingly, to control bacterial infectious diseases, it is difficult to analyse and develop novel antibiotic agents. Even though in present day medicinal studies has been evolved gradually by the scientific studies and modernization of techniques, then also the foundation of its growth remains deep rooted in ancient ayurvedic therapeutics and nutraceuticals.[3] Herbs are vital pharmacological prospective medium of antimicrobial agents in different parts of the world. In the developing countries about 60 to 90% of populations use plant-derived therapeutics. Plants are rich source of phytochemicals including phenols, tannins, alkaloids, terpenoids, and flavonoids proficiently leads to study *in-vitro* antimicrobial properties.[4]

***Corresponding Author:** Minakshi Pandey

Address: Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India

Email ✉**:** minakshi.res5@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Research into the antimicrobial activities of folk plant-based drugs over the last decade has been revisited and deeply analysed. In prevention and treatment of pathogenic diseases in human, medicines derived from natural resources play a significant role.[5] It has been estimated that solvent extracts from plants are potential sources of antiviral, anticancer and antimicrobial agents used in allopathic drugs.

Globally one of the major health issues is Oral diseases like dental caries, oral mucosal and periodontal diseases that is economically affecting public.[6] In developing countries 10% of Healthcare expenditure is related to dental hygiene and care. In most of the developed countries even though there is an enhancement in oral health sector, there are still with low socioeconomic status dentally deprived people.[7,8] Dental caries, has been diagnosed as one of 10th the most prevalent chronic oral conditions and is a major problem affecting 9% for human population worldwide.[9,10] The opportunistic chronic dental caries infection is caused by the colonization of pathogenic microorganisms, improper nutritional habits, and inappropriate oral hygiene leads to destruction and demineralization of hard tissues of teeth by acid production occurring from bacterial fermentation of food.[11,12] Oral *streptococci* are the first cariogenic isolated species to play a vital role in the dental plaque biofilm formation and blooming of caries. There have been four main factors in dental caries' etiology: bacteria, time, susceptible tooth surface, and fermentable carbohydrates. Due to the high prevalence of oral disease and increased microbial resistance against antibiotics, there is a need for alternative methods.[13,14] In spite of latest technology to diagnose and treat this oral condition, there is a high rise in prevalence of dental caries in India. *Streptococcus mutans, S. constellatus, S. sanguis, S. salivarius, S. sobrinus, S. mitis, S. anginosus, S. gordonii, S. intermedius, and S. oralis* are some of the primary acid-tolerant species of cariogenic bacteria that are associated with dental plaque.[15,16] These cariogenic pathogens are primary etiologic factor for dental caries, leading to colonization of tooth surfaces to initiate plaque formation and maturation through their ability to synthesize and bind extracellular polysaccharides (glucan) using the enzyme glucosyltransferase.[17] They also take up demineralization of inorganic tooth structure by metabolizing sucrose to lactic acid. The primary habitats for cariogenic pathogens are mouth, pharynx, heart and intestine. Therefore, several chemical factors, such as cohesion to enamel surfaces, acidic metabolites production, the ability to form glycogen reserves and the capacity of extracellular polysaccharides synthetization are present in dental caries.[18,19] Several antibiotics and antimicrobial agents have been used to eradicate cariogenic bacteria from the oral microflora. However, their clinical frequent use is restricted due to unenviable side effects including tooth staining, bacterial susceptibility, vomiting and diarrhoea.[20] These problems necessitate the importance of further advanced research to reinforce an alternative antibacterial nutraceutical from plant-based sources with an aimed focus on well-being of human health and effectiveness in the therapeutics of dental caries.[21]

The *P. granatum* L., is more often known as pomegranate, an ancient traditional remedial ayurvedic herb belongs to family *Punicaceae* is a deciduous shrub, regarded as "healing nutraceutical" due to its stupendous therapeutic effect, as all parts of pomegranate is having incredible ethnomedicinal efficacy for ailing innumerable pathogenic diseases. Punica, the genus name, was derived from the roman name for Carthage. The word Pomegranate means Apple ("pomum") and seeded ("granatus"). Pomegranate tree is the oldest domesticated tree for its countless health benefits, known even before the 21st Century. Pomegranate, considered "a pharmacy unto itself" herb contains gallic acid, anthocyanins, ellagic acid, glucose, catechin, ascorbic acid, caffeic acid, epigallocatechin, quercetin, rutin, iron and amino acids possessing anti-atherosclerotic, antihypertensive, antiaging, antimicrobial and antioxidative properties.[22] Systematic numerous research studies have identified and evaluated around 153 phytochemicals and their derivatives in pomegranate.[23] Epidemiological studies suggest that a reduced risk of cancer is associated with the consumption of a phytochemical- rich diet that includes fruits and vegetables. According to Badria *et. al.*, *P. granatum* L. flavonoids have shown antibacterial action *in vitro* against microbes causing gingivitis and probable reason for sensitivity of Oral pathogenic Streptococci to pomegranate extract are the tannins which increase bacteriolysis.[23] In a recent research study, according to Acharya *et al*. It is potentially demonstrated that pomegranate mouthwash had antibacterial effect against most important periodontal pathogens *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia*. Retting *et al.*, reported that the pomegranate fruit peels have chemo preventative capability due to presence of polyphenols via demonstration of inhibitory activities to various tumours.[24] As per Vasconcelos *et al.*, study, extract of *P. granatum* L. can be used to control the adhesion of different microbes in the oral cavity. They used various extracts of *P. granatum* against the streptococci strains, *S. mutans, S. mitis, C. albicans* and potent results were found against selected microbes.[25]

Thus, indigenous *P. granatum* L. peel, an ayurvedic Phyto-therapeutic agent as an effectual alternative to multi drug-resistant antibiotics in India, is being extensively evaluated today and is considered to be considered play a significant role in human healthcare nutraceutical.[26,27] Although, up to now, very few research investigations have been conducted on the antimicrobial activity of *P. granatum* L. peels against cariogenic bacteria. Since the primeval times, the *P. granatum* L. has been perceived

as medicinal nourishment with several beneficial effects in numerous human diseases.[28] Hence, considering the prevalence of dental diseases due to oral cariogenic pathogens and the recent interest in ayurvedic medicinal herbs, the present research study has focused on assessing the bio profiling of antibacterial activity of the ethanolic, aqueous and chloroform extracts of *P. granatum* L. peel *in vitro* against different oral cariogenic pathogens *Streptococcus mutans, S. sanguis, S. salivarius, S. sobrinus, S. mitis.*[29,30] In this study, we found that the comparatively crude ethanol extract isolated from pomegranate peels (PEP) exhibited a better inhibitory activity against caries causing pathogens than crude aqueous and chloroform extract isolated from pomegranate peels.

Materials and Methods

Collection and Identification Plant Material

For the experimental study, the fruits were obtained in March 2017 at a public market in Bengaluru, Karnataka, India. The fruits were brought to School of Biotechnology, Research and Product Development laboratory, GPS Institution of Agricultural Management, Bengaluru for identification, and identified as the *P. granatum L*. "Bhagwa" variety. Fruits were washed throughout with distilled water, and with tissue paper, surface water was wiped. Manually fruits were peeled out and peels were separated peels from arils.[31] Further peels were dried in an incubator at 33°C for 7 days and powdered using electric grinder. Ampicillin, was obtained from Hi-Media Laboratories (P) Ltd.

Preparation of Aqueous, Ethanol and Chloroform extract of *P. granatum L.*

The process of separating active plant materials or secondary metabolites from inert or inactive material using an appropriate solvent and standard extraction procedure is a crucial and key step to achieve quality result outcome.[32] The earlier studies conducted on various parts of *P. granatum* L solvents like Water, Ethanol and Chloroform were selected for plant extraction.[12,20,22,33-35] Peels of *P. granatum* L. was washed thoroughly with double distilled water. To obtain Aqueous extract, 50 gm of peels were homogenized with 100 mL boiling hot water using pestle and mortar, 400 mL of hot/cold water was mixed with the residue and kept for stirring for 30 minutes. The pooled extract was centrifuged at 10,000 rpm for 15 minutes at 40°C. Collected supernatant was concentrated by freeze drying using lyophilizer. The extract obtained was called as APG (Aqueous extract of *P. granatum L.*). Extract was filtered through 0.22-micron filter and stored at 20°C for further use. The dried extract was precisely weighed, labelled, and refrigerated. For Ethanol and Chloroform extracts 50 gm of peels weighed separately and were homogenized with 500 mL of each ethanol and chloroform using mortar and pestle. These were centrifuged at 10000 rpm for 10 minutes. Clear supernatant was concentrated using rotary evaporator at 38°C to 40°C. The extracts were dissolved in ethanol and chloroform respectively and kept in -20°C for further use.

Activation of Cariogenic Microorganisms

Standard strains of cariogenic microorganisms *S. sanguinis* (ATCC 10556), *S. mutans* (ATCC 25175), *S. salivarius* (ATCC 13419), *S. sobrinus* (ATCC 33402), and *S. mitis* (MTCC 497) were obtained in lyophilized form from the Hi-Media Laboratories Pvt Ltd and Micro Type Culture Collection and gene bank (MTCC). Cariogenic Bacterial strains were activated by inoculation in the brain heart infusion agar (BHIA) culture medium followed by 24 hours of incubation at 37°C. A 24 hours culture was used for preparation of microbial suspension. The concentration of microorganisms in the microbial suspension was adjusted to 0.5 McFarland standard using a spectrophotometer at a wavelength of 625 nm (a McFarland standard is a chemical solution with a turbidity comparable to that of microbial suspension. Using this suspension, number of bacteria per each millilitre of the suspension can be estimated, which is equal to 1.5 × 108 CFU/mL).

Preliminary Phytochemical Screening of *P. granatum L.*

Phytochemical examinations for *P. granatum L.* peels were carried out for all the extracts as per the standard methods as shown in Table 1. Chemicals and Reagents Solvents Millon's reagent, Benedict's reagent, Fehling's solution-A and B, gelatine powder, bromine water, sulphuric acid, ferric chloride and glacial acetic acid were purchased from Hi-Media Laboratories Pvt. Ltd.

Establishment of Antibacterial Activity Assay

Primary assessment of the antimicrobial effect of extracts was done using the means of the well diffusion technique. 500 μL of each microbial suspension at 0.5 McFarland standard concentration was cultured in BHIA (swabbed on the plate). The sterile BHI agar medium (20 ml) in Petri dishes was uniformly smeared using sterile cotton swabs with test pure cultures of human cariogenic bacteria *S. sanguinis, S. mutans, S. salivarius, S. sobrinus and S. mitis*. Then, wells measuring 8mm in diameter were created on the agar surface. Different concentrations of the extracts were prepared by serial dilution (dilution by one-half) using sterile distilled water solvent; 100 μL of each concentration of extract was poured into each well. The plates were incubated at 37°C for 24 hours. The time of contact for each solvent extracts were considered as 24 hours. The diameter of the growth inhibition zone was measured in millimetres. This process was repeated in triplicate and the mean diameter of the growth inhibition zone was calculated for different concentrations of the extract.

Table 1: Qualitative phytochemical analysis of *Punica granatum* L. peel extracts

S No.	Phytochemical constituent	Peel extract			Fruit extract		
		Aqueous	*Ethanol*	*Chloroform*	*Aqueous*	*Ethanol*	*Chloroform*
1.	Alkaloid	+	_	_	_	+	+
2.	Amino acid(free)	_	_	_	_	_	_
3.	Anthocyanins	_	_	_	_	_	_
4.	Betacyanin	+	+	+	+	+	+
5.	Carbohydrate	+	+	+	+	+	+
6.	Coumarin	+	+	+	+	+	+
7.	Flavonoids	+	+	+	+	+	+
8.	Glycosides	+	_	_	_	+	+
9.	Phenol	+	+	+	+	+	+
10.	Protein	+	+	+	+	+	+
11.	Saponin	_	_	_	+	+	+
12.	Steroids	_	_	_	_	+	+
13.	Tannin	_	+	+	_	+	+
14.	Terpenoids	_	_	_	_	+	+
15.	Vitamin C	+	_	_	_	+	+
16.	Quinones	+	+	+	+	+	+

'+'=Presence, '-'=Absence

Minimum Inhibitory Concentration

The MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism (0.5 McFarland standard in this study). It is the minimum concentration of the extract that completely prevents visible growth and multiplication of bacteria compared to the negative control group. To determine MIC, macro dilution method according to the standard technique described by the clinical and laboratory standards institute (CLSI) was used. Different concentrations of the extract were prepared by serial dilution (dilution by one-half) in BHI broth medium. Using this medium, microbial suspension was then diluted by one-half using the culture medium and 1mL of it was added to the tubes containing serially diluted extract. The negative control tube only contained the culture medium and extract with no microbial suspension. The positive control tube contained culture medium and microbial suspension with no extract. After 24 hours of incubation at 37°C, growth and proliferation of microorganisms were evaluated and the MIC value of the extract for each bacterial strain was determined. This test was repeated in triplicate for each microorganism.

Minimum Bactericidal Concentration

After determination of MIC, 20 μL of the suspension in the tube containing MIC of the extract and tubes showing no bacterial growth were cultured on plates containing BHIA. After 24 hours of incubation at 37°C, the plates were evaluated for growth of microorganisms. The concentration with no bacterial growth was determined as MBC. This test was repeated in triplicate for each microorganism. The effect of 0.2% Ampicillin on the microorganisms was also evaluated using the disk diffusion method and the MIC and MBC values of Ampicillin for the microorganisms were determined as well.

Statistical Analysis

All values were analysed that obtained in zone of inhibition measuring experiment for statistical significance by one-way analysis of variance (ANOVA) using statistical software GraphPad prism version 8.0. The graphs were also derived using the above-mentioned software. The data were demonstrated as Mean ± SEM of three trials. A P value < 0.05 was considered significant.

The present study results obtained in MIC and MBC were interpreted for statistical significance using one-way Analysis of Variance (ANOVA) and variations among means were compared using Post Hoc Duncan's Multiple Range test at $P \leq 0.05$ was considered significant [SPSS version 11.5.0 (SPSS Inc., Chicago, IL)].

Results

The present research of preliminary phytochemical analysis of *P. granatum* L. peels and arils are very significant in finding chemical metabolites that may lead to their quantitative assessment and also in locating the derived source of pharmacologically bioactive chemical compound.[34-35] Due to the presence of a wide range of phytochemicals in peels and arils of *P. granatum L.* plant, it can be estimated that they have significant Ethnomedicinal values.[36] Based

on primary phytochemical analysis experimental study shown in Table 1, pomegranate peels and arils contained certain important derivatives like phenols, tannins, flavonoids, quinones, coumarins, steroids, triterpenoids, alkaloids, proteins, glycoside, carbohydrate, betacyanin and saponin.[37] With scrutinizing systematic research and assessment its evaluated that phytochemical metabolites constitute essential therapeutical properties for human health and diseases.[38] These bioactive compounds presence with high potentiality substructures further antibacterial study in different extracts of *P. granatum L.* peels to affirm nutraceutical efficacy for ailing various human dental pathogenic diseases. Additionally, Crude extracts *P. granatum L.* peels were extracted with water, ethanol and chloroform. After estimating the antibacterial effects of crude aqueous extract, ethanolic extract and crude chloroform extract of *P. granatum L.* peels, the positive control produced remarkably large inhibition zones for all microorganisms and the negative control showed no notable inhibitory effect.

The mean (and SD) diameter of the growth inhibition zone due to the effect of crude ethanolic extract, crude aqueous extract, crude chloroform extract of *P. granatum* peels against *S. mutans, S. sanguinis, S. salivarius, S. sobrinus S. mitis* is shown in Figs. 1 to 5.

The crude ethanolic extract of *P. granatum L.* peels, showed high potent inhibitory effects on the growth and proliferation of all five bacteria *Streptococcus mutans, S. sanguis, S. salivarius, S. sobrinus, S. mitis* using the well diffusion technique. The largest diameter of growth inhibition zone belonged to *S. sanguinis, S. mitis and* the smallest zone shown by *S. salivarius* respectively. The crude aqueous extract of *P. granatum L.* peels, showed intermediate potent inhibitory effects on the growth and proliferation of all five bacteria *Streptococcus mutans, S. sanguis, S. salivarius, S. sobrinus, S. mitis* using the well diffusion technique. The largest diameter of growth inhibition zone belonged to *S. mitis*, *S. sobrinus and* the smallest shown by *S. salivarius* respectively. The crude chloroform extract of *P. granatum L.* peels,

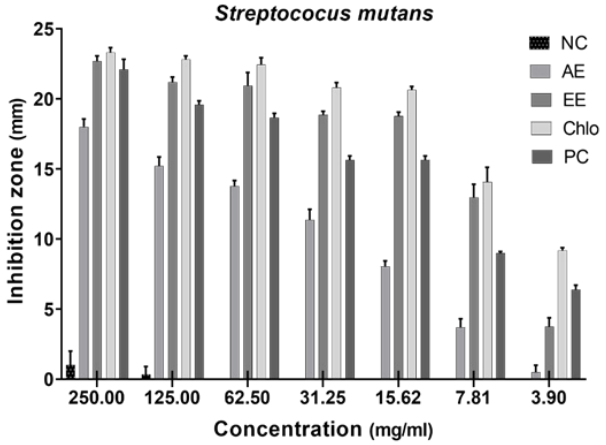

Fig. 1: Efficacy of various crude extracts of *P. granatum* peel on *S. mutans.* NC-Negative Control, AE-Aqueous Extract, EE-Ethanol Extract, Chlo-Chloroforrn, PC-Positive Control

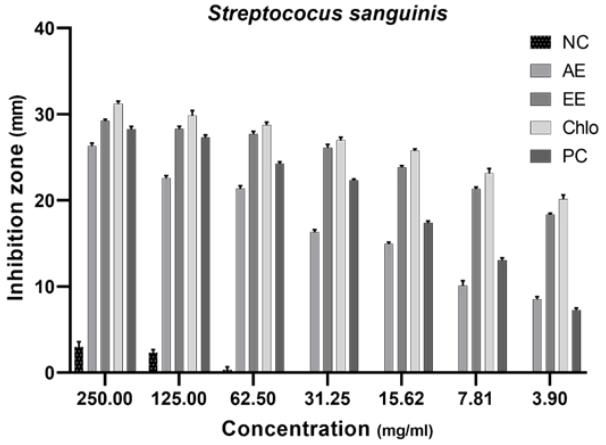

Fig. 2: Efficacy of various crude extracts of *P. gtanaturri* peel on *S. sanguinis*_ NC-Negative Control, AE-Aqueous Extract, EE-Ethanol Extract, Chlo-Chloroform, PC-Positive Control

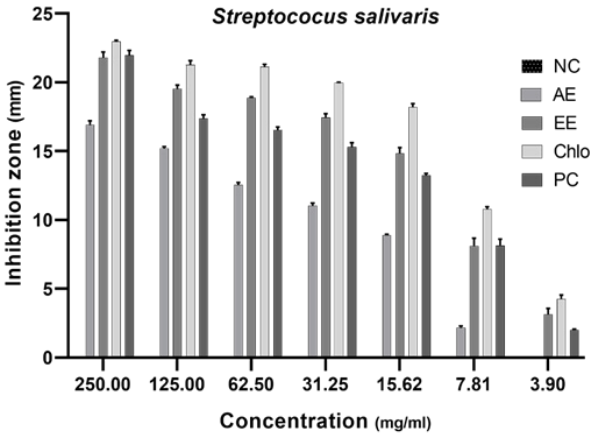

Fig. 3: Efficacy of various crude extracts of *P. granatum* peel on *S. salivaris*. NC-Negative Control, AE-Aqueous Extract, EE-Ethanol Extract, Chlo-Chloroform, PC-Positive Control

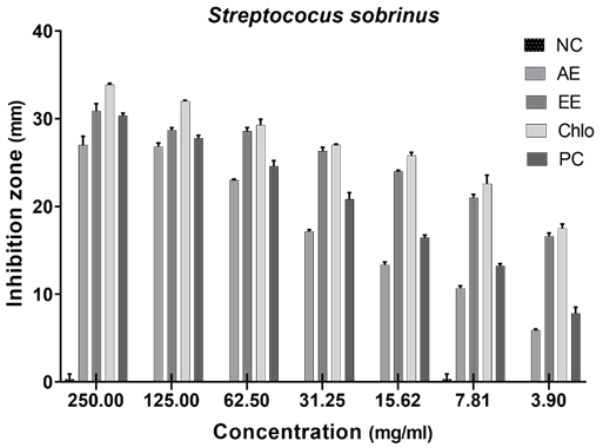

Fig. 4: Efficacy of various crude extracts of *P. granator* peel on *S. sobrinus*. NC-Negative Control, AE-Aqueous Extract, EE-Ethanol Extract, Chlo-Chlorotomi, PC-Positive Control

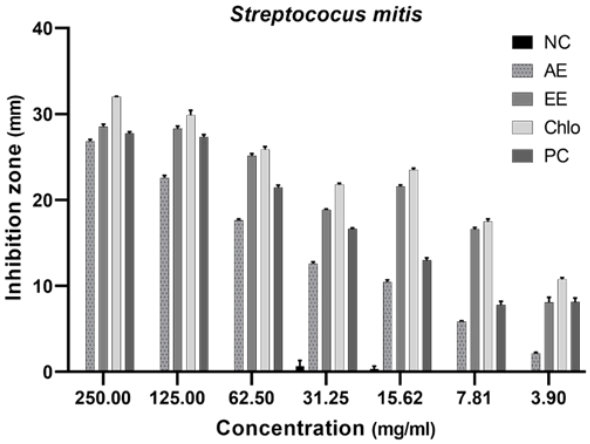

Fig. 5: Efficacy of various crude extracts of *P. granatum* peel on *S. mitis*. NC-Negative Control, AE-Aqueous Extract, EE-Ethanol Extract, Chlo-Chloroform, PC-Positive Control

showed high potent inhibitory effects on the growth and proliferation of all five bacteria *S. mutans, S. sanguis, S. salivarius, S. sobrinus, S. mitis* using the well diffusion technique. The largest and the smallest diameter of growth inhibition zone belonged to *S. sobrinus* and *S. salivarius*, respectively.

The MIC and MBC of crude aqueous extract, ethanolic extract and crude chloroform extract of *P. granatum L.* peels were determined using serial dilution method shown in Tables 2 and 3.

It was found that all the crude extract of *P. granatum L.* peels inhibited oral microbes *Streptococcus mutans, S. sanguis, S. salivarius, S. sobrinus, S. mitis*. In addition, the concentration of 3.9 mg/mL was the most effective crude ethanolic extract against *S. sanguinis* compared with other cariogenic *streptococci*. Further the concentration of 15.62 mg/mL was the most effective crude aqueous extract against *S. sanguinis* compared with other cariogenic streptococci as well the concentration of 7.81 mg/mL was the most effective crude aqueous extract against *S. sanguinis* compared with pathogens.

The ethanolic extract of medicinal plant *P. granatum L.* peels had MBCs of 3.9–31.25 mg/mL against *Streptococcus mutans, S. sanguis, S. salivarius, S. sobrinus, S. mitis.* The crude ethanolic extract of *P. granatum L.* peel displayed the most outstanding in-vitro antibacterial activity with MBC of 3.9 mg/mL against *S. sanguinis.* Additionally, MBC values revealed that the crude aqueous extract had the highest effect on *S. sanguinis* of 31.25 mg/mL compared with other cariogenic streptococci. Furthermore, crude chloroform extract shown that highest potency against *S. sanguinis* at the concentration of 7.81 mg/mL in comparison to other cariogenic streptococci under study.

The highest antibacterial effect of *P. granatum L.* peel crude ethanolic extract was on *S. sanguinis* with MIC and MBC values of 3.9 mg/mL and lowest antimicrobial activity on *S. Salivaris.* Hence dental caries pathogens were found to be resistant against crude ethanolic, aqueous and chloroform extracts of *P. granatum L.* peel.

Table 2: The MIC (mg/mL) of *P. granatum* L. peel extracts for different cariogenic bacteria

Pathogens	*AE*	*CF*	*EE*
S. salivaris	125	62.5	15.62
S. mutans	31.25	15.62	7.81
S. sanguinis	15.62	7.81	3.9
S. sobrinus	62.5	31.25	15.62
S. mitis	31.25	15.62	7.81

Table 3: The MBC (mg/mL) of *P. granatum* L. peel extracts for different cariogenic bacteria

Pathogens	*AE*	*CF*	*EE*
S. salivaris	NS*	125	31.25
S. mutans	62.5	15.62	15.62
S. sanguinis	31.25	7.81	3.9
S. sobrinus	62.5	31.25	15.62
S. mitis	125	31.25	15.62

*Not Seen

Discussion

The pivotal and substantial utilization of antibiotics are prevailing worldwide in clinical therapeutics that further lead to nurturing the development of antibiotic resistances among pathogenic microbes and in due course of time it reflects a major problem in the curing of disease causing microorganisms, this has outpaced the origination of new alternative antimicrobial sources mainly from plants with the purpose to discover new bioactive therapeutics comprising bioactive compounds which overpower the multi drug resistance phenomenon.[39] Now a days, traditional herbal medicines are frequently used for healthcare in both developed and developing countries. Globally natural products are still one of the vital sources of new drug nutraceutical, that have been used for centuries to treat infectious diseases. Secondary metabolites are the bioactive compounds, produced by plants and due to their astounding structural diversity are widely used in the pharmaceutical industry and array of phytomedicinal activities.[40,41] Diverse works have been done to estimate the enormous medicinal effects of traditional plants extracts, including roots, stem, leaves or flowers in primary health care.

Worldwide dental caries is a one of the most susceptible and common oral multi-factorial pathogenic disease other than periodontal disease caused by acids from bacterial metabolic activity diffusing into enamel and dentine.[42] Cariogenic bacterial plaque commonly described as accumulation of microbes (mainly bacteria) rooted in polymer matrix of salivary and bacterial origin on tooth

surfaces which plays a pivotal role in the pathogenicity of periodontal disease and dental caries.[43-44] The first caries forming species are oral streptococci like *Streptococcus mutans, S. sanguinis, S. salivarius, S. sobrinus, S. mitis*. Amid all cariogenic microbes *Streptococcus sanguinis* is the first superintend for emergence, proliferation and maturation of plaque. *S. salivarius* is a prevalent colonizer of oral mucosal surfaces especially in the dorsal aspect of the tongue, buccal mucosa, and saliva. *Streptococcus mutans* have a vital role in growth, development and maturation of caries. The cariogenic properties comprised by oral bacteria are accredited to the production of indissoluble glucans from sucrose, their acidogenicity and their potentiality to adhere to tooth surfaces.[45] Enormous preventive and inhibitory strategies have been set out and examined for the termination of dental caries entirely and the research studies still goes on. In addition to this critical pathogenicity, most antibacterial agents can also nurture the occurrence and growth of resistant bacterial strains. [46-47] Hence, it becomes a prerequisite for the present therapeutic salubrious research to evaluate naturally available bioactive compounds which are invulnerable for humans and precise for dental caries, due to their insubstantial side effects and the patient is treated with determinedly. Globally this traditional therapeutic approach is of great significance especially in India due to the endemic occurrence and development of infective dental caries. More recently, to reduce the prevalence of dental caries a number of scientific medicinal research demonstrated the potential antibacterial properties of various ayurvedic herbs as an ethno-nutraceutical.[48] One of the most potent traditional plant is *P. granatum L.* has been used for thousands of years as holistic herb to treat a broad range of pathogenic diseases across different parts of the word.

P. granatum L. (Pomegranate) belonging to family *Punicaceae*, is a traditional ayurvedic medicinal herb, has long been reckoned as nutrient diet and Phyto drug, it is vividly used in Ayurveda, Siddha and Unani medicine especially for the ailing of numerous pathogenic diseases in human. *P. granatum L.* is a primeval traditional plant considered important and is familiar to have been cultivated more than 5,000 years ago in the Middle East region.[48] *P. granatum L.* is grown in many Mediterranean countries Middle Eastern, South Asian, and the USA, although Iran, China, India and Turkey are popular producers. Different parts of *P. granatum L.* contain a variety of phytochemicals like alkaloid tannins, flavonoids and polyphenols is well studied and could be a potent nutraceutical in treating of several pathogenic illnesses.

The present research study was focused analytically to determine the potential antibacterial effect of crude ethanolic, aqueous and chloroform extracts of *P. granatum L.* peels on cariogenic bacteria *Streptococcus mutans, S. sanguinis, S. salivarius, S. sobrinus, S. mitis in-vitro* using the well diffusion method. The growth inhibition zone diameter, MIC and MBC values of the crude extracts for different microbes were also calculated and compared to positive control Ampicillin.[49-50] Based on the results, the crude extracts of *P. granatum L.* peels had prophylactic effect on the origination, growth and proliferation of all five cariogenic bacterial strains. In the current study the MIC of *P. granatum L.* peel is evaluated and compared it with Ampicillin against adhesion of S. mutans, *S. sanguinis, S. salivarius, S. sobrinus* and *S. mitis*. Its shown that crude extracts of *P. granatum L.* peel was more effective in preventing the adhesion of streptococci. The highest antibacterial effect of *P. granatum L.* peel crude ethanolic extract was on *S. sanguinis.* Therefore, dental caries pathogens were found to be resistant against crude ethanolic, aqueous and chloroform extracts of *P. granatum L.* peel.

Kakiuchi *et al.* [51] evaluated the specific antimicrobial activity of *P. granatum L.* on dental biofilm bacteria, i.e., disturbance of polyglycan synthesis, thus acting on the assimilation and adherence mechanisms of these microbes to dental surface. The present experiment demonstrated the inhibitory effect of adherence of a gel derived from *P. granatum L.,* which is a fruit rich in phytochemicals like tannins and polyphenols. The results supported that the glucan synthesis and its antimicrobial effect of gel proved as a potent control of the already developed biofilm, which is considered the primary preventative agent in periodontal, caries disease and stomatitis.

In an experimental demonstration by Vahid-Dastjerdi *et al.,*[52] the potency of water extract of *P. granatum* flower (petal) on the oral streptococci as in our present assessment was estimated. The highest antimicrobial effect was seen on *S. sanguinis* compared to the MIC and MBC values obtained in the current study. Hence, it analysed that the solvent containing alcohol constitutes more antimicrobial bioactive compounds and consequently shows high antimicrobial efficacy on *S. mutans*.

The influence of antimicrobial properties to fight against various cariogenic bacterial infections have been reported in several research investigations. In correspondence to Mezouar *et al.* [53] methanolic extracts of root barks of *B. vulgaris* have presented a very weak antibacterial effect against all tested microbial strains including *S. aureus*. In comparison to literature research estimations the results found in this study were noticed as potential antimicrobial activity of some natural therapeutic plants from Tunisia. Methanolic extracts of *C. monspeliensis* leaves have shown an interesting effect against *P. aeruginosa, S. aureus, E. faecalis* with inhibition zones respectively. While water-methanol extracts of fruit peels of pomegranate (*P. granatum*) have exhibited adequate activity when tested on *S. aureus, P. aeruginosa and K. pneumoniae*. This potential of pomegranate peels could be attributed to presence of bioactive metabolite like tannins, for which antimicrobial activity evaluation studies has been done.

One principal element influencing the MIC is the variance in the composition of crude extracts of traditional herb due to impact of the geographical orientation of the therapeutic fauna of herb, senescence of plant, developing stage, harvesting conditions and season, technique of drying, and extraction procedure. Also, crude extracts of distinct parts of the ayurvedic herb have varying level of antimicrobial effect and cariogenic bacteria have variable sensitivity to different extracts of the plant. Alcoholic extract of *P. granatum* has notable isolated bioactive metabolite like tannins, phenols, alkaloids, flavonoids, gamma terpinene, steroids, betacyanins, coumarin, proteins. Hence all previous research investigations have manifested the considerable antimicrobial efficacy of *P. granatum L.*, although further much assessment required on therapeutic potency of bioactive compounds.

In conclusion of the present experiential study, these in vitro antimicrobial results suggest the potency of *P. granatum* peel extract in dental caries. Use of more than one *P. granatum* plant part, even a combination of parts for the formulation can come as a potent controlling agent to complete cure of dental caries. Peel extracts viewing antimicrobial activity with minimum inhibitory concentration show the efficacy of the plant products that could be considered as a good indicator of prospective plants for discovering new antimicrobial agents against dental caries pathogens. The interpretations were drawn that Crude ethanolic, aqueous and chloroform extracts of *P. granatum* peels would be beneficial bioactive compounds for the generation of antibacterial agents as a formulation against *S. mutans, S. sanguinis, S. salivarius, S. sobrinus and S. mitis,* however, the latter requires higher concentration of the crude extracts to attain MIC and MBC. Although few cariogenic bacteria efficacies were less than Ampicillin, they may have a prospective role for dental caries interception. Consequently, the present research investigations could potentially exhibit a scientific foundation for the traditional ayurvedic therapeutics of *P. granatum* L. peels on oral and dental pathogenic microorganisms, shelling out in improvement of oral health and hygiene along with inhibiting the complications and reducing cost of the treatment with synthetic medicine. Though, additional clinical trials studies seem essential to analyse their efficacy and benefits to human infectious diseases as well as environment. Henceforth the findings of this study provide a potential lead in medical sciences to further development of nutraceutical formulations from *P. granatum* for the therapeutic of dental caries. It is concluded that the crude extracts of *P. granatum peels* shows as a potential ethnonutraceutical efficacy against cariogenic *streptococci*.

Acknowledgements

The authors are appreciative and grateful to the School of Biotechnology, GPS Institute of Agricultural Management, Bengaluru, Karnataka, India for constant encouragement during this study and providing facilities to conduct research investigations.

References

1. Alviano WS, Alviano DS, Diniz CG, Antoniolli AR, Alviano CS, Farias LM, *et al*. *In vitro* antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Arch. Oral Biol. 2008; 53:545–552.
2. Saraya S, Kanta J, Sarisuta N, Temsiririrkkul R, Suvathi Y, Samranri K. Development of guava extract chewable tablets for anticariogenic activity against *Streptococcus mutans*. Mah Univer Jou of Pharma Sci. 2008;35(1-4):18–23
3. Perez C, Anesini C. *In vitro* antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. J. Ethnopharmacol. 1994; 44:41-46
4. Hintao J, Teanpaisan R, Chongsuvivatwong V, Ratarasan C, Dahlen G. The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without Type 2 diabetes mellitus. *Oral. Microbiol. Immunol.* 2007;22(3):175–181.
5. Rhodes DV, Crump KE, Makhlynets O, *et al*. Genetic characterization and role in virulence of the ribonucleotide reductases of *Streptococcus sanguinis*. J. Biol. Chem. 2014;289(9):6273–6287
6. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug and resistant human pathogens. J. Ethnopharmacol. 2001; 74: 113-123.
7. Singh VP, Sharma SK, Khare VS. Medicinal plants from Ujjain District Madhya Pradesh, Part II. Indian Drgs Pharm. Ind. 1980; 5: 7-12.
8. Arseculeratne SN, Gunatilaka AAL, Panabokke RG. Studies on medicinal plants of Sri Lanka. Part 14. J. Ethnopharmacol. 1985; 13: 323-335.
9. Dziedzic A, Kubina R, Wojtyczka RD, Kabala-Dzik A, Tanasiewicz M, Morawiec T. The antibacterial effect of ethanol extract of *Polish propolis* on mutans streptococci and lactobacilli isolated from saliva. Evid. Based Complement. Alternat. Med. 2013; 681891. doi:10.1155/2013/681891.
10. Hamilton-Miller JM. Anti-cariogenic properties of tea (*Camellia sinensis*). J. Med. Microbiol. 2001;50, 299–302. doi:10.1099/0022-1317-50-4-299.
11. Burapadaja S, Bunchoo A. Antimicrobial activity of tannins from Terminalia citrina. Planta medica. 1995 Aug;61(04):365-366.
12. Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP, Pal M. Studies on antidiarrheal activity of *Punica granatum* seed extract in rats. Journal of Ethnopharmacology. 1999; 68: 205-208.
13. Park SJ, Han KH, Park JY, Choi SJ, Lee KH. Influence of bacterial presence on biofilm formation of *Candida albicans*. Yonsei Medical Journal. 2014; 55:449–458.
14. Byun R, Nadkarni MA, Chhour KL, Martin FE, Jacques NA, Hunter N. Quantitative analysis of diverse *Lactobacillus* species present in advanced dental caries. J Clin Microbiol. 2004;42:3128–36.
15. Nishijima T, Teruya K, Yanase M, Tamori Y, Mezaki K, Oka S. Infectious endocarditis caused by *Lactobacillus acidophilus* in a patient with mistreated dental caries. *Internal Medicine.* 2012;51(12): 1619–1621.
16. Balhaddad AA, Kansara AA, Hidan D, Weir MD, Xu HHK, Melo MAS. Toward dental caries: exploring nanoparticle-based platforms and calcium phosphate compounds for dental restorative materials. *Bioactive Materials.* 2018;4:43–55.
17. Weng Y, Guo X, Chong VJ, Howard L, Gregory RL, Xie D. Synthesis and evaluation of a novel antibacterial dental resin composite with quaternary ammonium salts. *Journal of Biomedical Science and Engineering*. 2011;4(3):147–157. doi: 10.4236/jbise.2011.43021
18. Ruiz-Linares M, Ferrer-Luque CM, Arias-Moliz T, de Castro P, Aguado B, Baca P. Antimicrobial activity of alexidine, chlorhexidine and cetrimide against *Streptococcus mutans* biofilm. *Annals of Clinical Microbiology and Antimicrobials.* 2014;13:41.

19. Badria FA, Zidan OA. Natural products for dental caries prevention. J Med Food. 2004;7:381-4.
20. Ozkan M. Degradation of anthocyanins in sour cherry and pomegranate juices by hydrogen peroxide in the presence of added ascorbic acid. Food Chem. 2002; 78:499–504.
21. Zhicen L. Colour Atlas of Chinese Traditional Drugs. Vol II. Science Press, Beijing, People's Republic of China, 1987, p 75-76.
22. Pai MB, Prashant GM, Murlikrishna KS, Shivakumar KM, Chandu GN. Antifungal efficacy of *Punica granatum*, *Acacia nilotica, Cuminum ciminum* and *Foeniculum vulgare* on *Candida albicans*: An *In vitro* study. Indian J Dent Res. 2010; 21:334–6.
23. Babu J, Blair C, Jacob S, Itzhak O. Inhibition of *Streptococcus gordonii* metabolic activity in biofilm by cranberry juice high-molecular-weight component. J. Biomed. Biotechnol. 2012;590384. doi:10.1155/2012/590384.
24. Hayati F, Okada A, Kitasako Y, Tagami J, Matin K. An artificial biofilm induced secondary caries model for *in vitro* studies. *Australian Dental Journal. 2011;* 56(1):40–47.
25. Gonçalves FA, Andrade Neto M, Bezerra JN, Macrae A, Sousa OV, Fonteles-Filho AA, *et al*. Antibacterial activity of Guava, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob Shrimp, Xiphopenaeus kroyeri (Heller). Rev Inst Med Trop Sao Paulo. 2008; 50:11–5.
26. Varasteh F, Arzani K, Barzegar M, Zamani Z. Changes in anthocyanins in arils of chitosan-coated pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) fruit during cold storage. Food Chem 2012; 130:267–272.
27. Jafri MA, Aslam M, Javed K, Singh S. Effect of *Punica granatum Linn.* (flowers) on blood glucose level in normal and alloxan induced diabetic rats. Journal of Ethnopharmacology. 2000; 70: 309-314.
28. Khan DM, Bernaitis L, Shobha KL, Ashok M, Shenoy RP. Antifungal activity of *Taxus baccata, Phyllanthus debilis, Plectranthus amboinicus* against *Candida* species of clinical origin. Int. J. Biol. Pharm. Res. 2013; 386-389.
29. Jannadi H, Correa W, Zhang Z, Brandenburg K, Oueslati R, Rouabhia M. Antimicrobial peptides Pep19-2.5 and Pep19-4LF inhibit *Streptococcus mutans* growth and biofilm formation. *Microbial Pathogenesis.* 2019; 133. doi: 10.1016/j.micpath.2019.103546.103546.
30. Huang X, Zhang K, Deng M, *et al*. Effect of arginine on the growth and biofilm formation of oral bacteria. *Arch. Oral. Biol.* 2017;82:256–262.
31. Giacaman RA, Torres S, Gómez Y, Muñoz-Sandoval C, Kreth J. Correlation of *Streptococcus mutans* and *Streptococcus sanguinis* colonization and *ex vivo* hydrogen peroxide production in carious lesion-free and high caries adults. *Arch. Oral. Biol.* 2015;60(1):154-9.
32. Abubakar A. R, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. J Pharm Bioall Sci 2020;12:1-10.
33. Dahham SS, Ali MN, Tabassum H, Khan M. Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum L.*). Am.–Eurasian J. Agric. Environ. Sci. 2010; 9:273-281.
34. Satomi H, Umemura K, Ueno A, Hatano T, Okuda T, Noro T. Carbonic anhydrase inhibitors from the pericarps of *Punica granatum L.* Biol Pharm Bull. 1993;16:787-790.
35. Rettig MB, Heber D, An J, Seeram NP, Rao JY, Liu H, Klatte T, Belldegrun A, Moro A, Henning SM, Mo D. Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-κB-dependent mechanism. Molecular Cancer Therapeutics. 2008 Sep 1;7(9):2662-2671.
36. Manasathien J, Kupittayanant S, Indrapichate K. Protective efficacy of pomegranate *(Punica granatum Linn., Punicaceae*) peel ethanolic extracts on UVB-irradiated rats kin. American–Eurasian Journal of Toxicological Sciences. 2011;3 (4):250–258.
37. Tehranifar A, Selahvarzi Y, Kharrazi M, Bakhsh V. High potential of agro- industrial by-products of pomegranate (*Punica granatum L.*) as the powerful antifungal and antioxidant substances. Industrial Crops and Products. 2011; 34:1523–1527
38. Yoshimura M, Watanabe Y, Kasai K, Yamakoshi J, Koga T. Inhibitory effect of anellagic acid-rich pomegranate extract on tyrosinase activity and ultraviolet-induced pigmentation. Bioscience, Biotechnology, and Biochemistry. 2005;69:2368–2373.
39. Ouachrif, A., Khalki, H., Chaib, S., Mountassir, M., Aboufatima, R., Farouk, L., Benharraf, A., Chait, A. Comparative study of the anti-inflammatory and antinociceptive effects of two varieties of Punica granatum. Pharmaceutical Biology. 2012;50(4),429–438.
40. Singh YN. Traditional medicine in Fiji: Some herbal folk cures used by Fiji Indians. Journal of Ethnopharmacology. 1986; 15: 57-88.
41. Mestry SN, Gawali NB, Pai SA, Gursahani MS, Dhodi JB, Munshi R, Juvekar AR. *Punica granatum* improves renal function in gentamicin-induced nephropathy in rats via attenuation of oxidative stress. Journal of Ayurveda and integrative medicine. 2018; 11(1):16-23.
42. Al-Megrin WA. Efficacy of pomegranate (*Punica granatum*) peel extract against hymenolepis nana in infections mice. Biosciences Biotechnology Research Asia. 2016; 13:103-108.
43. da Silva JA, Rana TS, Narzary D, Verma N, Meshram DT, Ranade SA. Pomegranate biology and biotechnology: A review. Scientia Horticulturae. 2013 Aug 27;160:85-107.
44. Brighenti FL, Gaetti-Jardim Jr E, Danelon M, Evangelista GV, Delbem AC. Effect of Psidium cattleianum leaf extract on enamel demineralisation and dental biofilm composition in situ. Archives of oral biology. 2012 Aug 1;57(8):1034-1040. doi:10.1016/j.archoralbio.2012.02.009.
45. Vasconcelos LC, Sampaio FC, Sampaio MC, Pereira Mdo S, Higino JS, and Peixoto MH: Minimum inhibitory concentration of adherence of *Punica granatum Linn* (pomegranate) gel against *S. mutans, S. mitis* and *C. albicans*. Brazilian Dental Journal 2006; 17(3):223-227.
46. Menezes SM, Cordeiro LN, Viana GS. *Punica granatum* (pomegranate) extract is active against dental plaque. Journal of Herbal Pharmacology. 2006; 6(2):79-92.
47. Braga LC, Shupp JW, Cummings C, Jett M, Takahashi JA, Carmo LS, Chartone-Souza E, Nascimento AM. Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin production. J Ethnopharmacol. 2005; 4:96:335-409.
48. Ge X, Kitten T, Chen Z, Lee SP, Munro CL, Xu P. Identification of *Streptococcus sanguinis* genes required for biofilm formation and examination of their role in endocarditis virulence. *Infect. Immun.* 2008;76(6):2551–2559.
49. Hillman JD, Cransky SSS, Shivers M. The relationships between streptococcal species and periodontopathic Bacteria IN Human Dental Plaque. Arch Oral Biol. 1985;30:791–795.
50. Abdollahzadeh SH, Mashouf RY, Mortazavi H, Moghaddam MH, Roozbahani N, Vahedi M. Antibacterial and antifungal activities of *Punica granatum* peel extracts against oral pathogens. Journal of Dentistry. 2011;8(1):1–6.
51. Kakiuchi N, Hattori M, Nishizawa M. Studies on dental caries prevention by traditional medicines. Inhibitory effect of various tannins on glucan synthesis by glycosyltransferase from *Streptococcus mutans*. Chem Pharm Bull 1986;34:720-725.
52. Dastjerdi EV, Abdolazimi Z, Ghazanfarian M, Amdjadi P, Kamalinejad M, Mahboubi A. Effect of *Punica granatum* L. flower water extract on five common oral bacteria and bacterial biofilm formation on orthodontic wire. Iranian journal of public health. 2014 Dec;43(12):1688.
53. Mezouar D, Lahfa FB, Abdelouahid DE, Adida H, Rahmoun NM, Boucherit Otmani Z. Activité antimicrobienne d'extraits d'écorce de racines de Berberis vulgaris. Phytothérapie 2014;12(6):380-385.

How to Cite this Article: Pandey M, Kumar SR. Bio Profiling of Antibacterial Activity of *Punica granatum L.*: An Ethnonutraceutical Against Cariogenic *Streptococci*. Int. J. Pharm. Sci. Drug Res. 2021;13(5):448-456. DOI: 10.25004/IJPSDR.2021.130501