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IN VITRO ANTIMICROBIAL ACTIVITY OF PHYTOCHEMICAL EXTRACT OF *PUNICA GRANATUM* ON *STREPTOCOCCUS MUTANS*

Shobha Shrivastava and Nidhi Jaiswal

Barkatullah University, Bhopal - 462 026 (Madhya Pradesh), India.

Abstract

The mouth is full of bacterial micro flora, it provides perfect atmosphere for microbial growth. *Streptococcus mutans* is considered as a potent caries causing bacteria and most common caries causing pathogen isolated from dental plaque. The aim of the study is to determine the *in vitro* inhibitory potential of phytochemical extract of *Punica granatum* (its rinds) on *S. mutans*. The pomegranate (*Punica granatum*) is a fruit-bearing shrub or small tree in the family Lythraceae. It has very good antioxidant properties, we wanted to check its antimicrobial activity also. The method used for extraction of phytochemicals from pomegranate was soxlet extraction. Both Aquous and Ethanolic extracts was obtained and used for testing its antimicrobial activity against *S. mutans*. The percentage yield so obtained was 45% and 61%, respectively. The antimicrobial activity was performed by using well diffusion method. *S. mutans* was seeded on NAM (Nutrient agar media). Both aquous and ethanolic extract of *P.granatum* was filled in wells of 6mm diameter on NAM plate and incubated at 37 degree C for 24 hrs. The recorded zone of inhibition was found out to be 18mm and nil, respectively. Thus, this showed that *Punica granatum* in its aquous extract is a potent antimicrobial against *S. mutans*, which can be used in many formulations of mouthwashes, dental tubes etc as a potent constituent.

Key words : Antimicriobial activity, *in vitro*, *S. mutans*, aquous extract, *P. granatum*.

Introduction

The mouth has a very mixed bacterial flora, the abundant moisture and the constant presence of small food particles provide an ideal environment for bacterial growth (Stewart and Beswick, 1977). The composition and consistency of diet, oral hygiene, clinical health and the use of antimicrobial agents all play a part in determining the quantitative nature of the oral flora. Tooth decay is caused by specific types of acid-producing bacteria that cause damage in the presence of fermentable carbohydrates such as sucrose, fructose and glucose (Hardie, 1982; Holloway and Moore, 1983; Rogers, 2008). Dental caries is a localized and transmissible pathological infectious process that ends up in the destruction of the hard dental tissue (Rathod *et al*., 2012). Although, 200 to 300 bacterial species have been found in saliva, Streptococcus mutans has been considered as a potent caries causing bacteria (Kuramitsu, 2001). The cariogenecity of this bacterium is associated with various factors including dextran production, production of high concentration of acid in the plaque and glycosial transferage activity. As glycan is the main component of dental biofilm and is directly proportional to the production of glycosial transferage by *S. mutans*. Therefore, *S. mutans* is a most common pathogen isolated from human dental plaque and its prevalence has been reported (Straetemans *et al*., 1998; Okada *et al*., 2005). As a science of life and the world's oldest medical system, Ayurveda has a holistic approach to health and disease that focuses on preserving and promoting good health and preventing disease through healthy lifestyle practices (Cohen, 2014). Kukreja *et al*. (2012) stated that various herbal products and their extracts such as guava, pomegranate, neem, propolis, tulsi, green tea, cranberry, grapefruit etc. have shown significant advantages over the chemical ones. *Punica granatum* known as 'Annar' in Urdu and 'Pomegranate' in English is the famous edible fruit. In traditional medicine, it has been used for the treatment of various diseases in America, Europe, Africa and Asia. In addition topast uses, *P. granatum* is used in several medicines for a variety of ailments (Olapour and

Najafzadeh, 2010). The aim of the present study is to determine *in vitro* effective inhibitory action of phytochemical extract of *Punica granatum* against *S. mutans*.

Materials and Methods

Collection of samples

Punica granatum was obtained from market and *Streptococcus mutans* for present study was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

Extraction of Phytochemical & Screening

Punica granatum rinds after collection, identification and approval were subjected to cleaning, shade drying and pulverization into fine powder before the process of extraction starts.

1. Defatting of plant material

Before applying extraction, the powdered plant materials were defatted by soaking it in petroleum ether at room temperature for 24 hours to remove any fatty, oily or lipid content from them. After defatting of plant material the petroleum ether was remove by filtration and the crud drug is again dried that is to be extracted with distilled water and ethanol.

2. Soxhlet extraction

A. Aqueous extraction of Defatted Crud Drug

About 10-20 grams of defatted dried powder was subjected to soxhlet extraction with 200 ml of distilled water as extraction solvent. Soxhelation process was carried out till the complete exhaustion of sample material at 80°C. The defatted fine powders of *Punica granatum* rinds were subjected to soxhlet extraction with distilled water.

B. Ethanolic Extraction of Defatted Crud Drug

Like the aqueous extraction about 10-20 grams of defatted dried powder was subjected to soxhlet extraction with 200 ml of 65% ethanol as extraction solvent till the complete exhaustion of sample material at 65°C. The defatted fine powders of *Punica granatum* rinds were all subjected to soxhlet extraction with 65% ethanol.

C. Concentration of Extracted Drug

The extract so obtained after the process of soxhletion was subjected to evaporation of solvent to get the extract in form of crystals, slurry or paste. This is done by taking the extracted drug containing solvents in a glass beaker and placing them in a boiling water bath. The contents were kept in boiling water bath till the solvent of extract is evaporated completely. The phyto extracts so obtained after this are now could be used to assess the yield of phytochemical extraction, evaluation of organoleptic properties, phytochemical analysis and other biological or pharmacological studies.

Antimicrobial activity

The antimicrobial activity was performed using well diffusion method for which *S. mutans* was seeded on NAM (Nutrient agar media). Both aquous and ethanolic extract of *P. granatum* was filled in wells of 6mm diameter on NAM plate and incubated at 37^0C for 24 hrs to 48 hrs and results was noted.

Results and Discussion

When the aquous and ethanolic extracts of *P. granatum* (rinds) was tested for its antimicrobial activity against *Streptococcus mutans*, the recorded zone of inhibition was found out to be 18mm and nil, respectively. The results of this study is in conjunction with that of Lalwani *et al*. (2014), who in their study demonstrated that the pomegranate aril extract has an antimicrobial effect against *Streptococcus*, thus acting as an anti-cariogenic agent.

Conclusion

The continuous use of antiseptics and antibiotics not only are associated with the adverse effects on host including hypersensitivity, depletion of beneficial gut and mucosal microorganism, immune suppression and allergic reactions, but also the pathogens develops the resistance against the antibiotics (Lopez *et al*., 2000). This imposes the need to search and development of more promising and reliable new and effective alternative oral health protection products of natural origin with curative properties, but being safe. Plants are the important source of potentially useful structures for the development of new chemotherapeutic agents. Thus from the present study, it can be concluded that *P. granatum* has good antimicrobial effect against *Streptococcus mutans*, caries causing bacteria which could be utilized in many ways after further advance studies and proper data generation for the development of new chemotherapeutic agents.

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