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COMPARATIVE ANTIBACTERIAL STUDY OF DIFFERENT EXTRACT OF POMEGRANATE AND ITS WILD VARIETY

Archana Devi*[1], Virender Singh [2] and A. B. Bhatt [1]

Department of Botany and Microbiology, HNB Garhwal University [1], Srinagar- 246 174, Uttrakhand, India
Department of Microbiology, Himachal Institute of Life Sciences [2], Paonta Sahib- 173 025, Himachal Pradesh, India

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Correspondence to Author:

Archana Devi

Department of Botany and Microbiology, HNB Garhwal University (A Central University), Srinagar (Garhwal) - 246 174, Uttarakhand, India

ABSTRACT

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being. We report in this work for the first time, the potent antibacterial activity of *Punica granatum* and its wild form *daru.* Ethanol, petroleum ether and distilled water extract of fruit's white membrane were screened for *in vitro* activity against four dental bacteria- *Streptococcus sp., Lactobacillus sp*., *Staphylococcus sp.* and *Proteus sp.* This plant was selected due to its traditional use for the treatment of oral infections. Among three tested extracts ethanol extract was the most effective against all the four dental bacteria.

INTRODUCTION: The potential for developing antibacterial from higher plants appears rewarding as it will lead to the development of a phytomedicine, to act against microbes. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. In rural areas of the developing countries, they continue to be used as the primary source of medicine [1]. About 80% of the people in developing countries use traditional medicines for their health care [2]. In the 2000-2006, approximately 50% of new chemical molecules extracted from natural products demonstrated their importance for the development of drugs in the treatment of infectious diseases [3].

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-infective compounds. As there are approximately 500000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds.

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. Dental diseases are a type of infectious diseases caused by pathogenic bacteria in dental plaque. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases as they are a rich source of antimicrobial agents [4]. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopeia as agents used to

treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. The general antimicrobial activities of medicinal plants and plant products have been reviewed previously [5]. In particular, traditional medicinal plant extracts or phytochemicals that have been shown to inhibit the growth of oral pathogens, reduce the development of dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases will be discussed subsequently. Many clinical studies have investigated the safety and efficacy of such plant-derived medicines.

Punica granatum L. (Punicaceae) is a native shrub of occidental Asia and Mediterranean Europe, popularly referred to in English as pomegranate [6]. The activity of pomegranate extracts against dental bacteria have been studied for years, but in a more intensified way during the last three decades. During this period, numerous antimicrobial screening evaluations has been published based on the traditional use of Chinese, African and Asian plant based drugs [7]. Extracts of all parts of the fruit appear to have therapeutic properties [8].

Most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins) [9], punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones. The peel of *Punica granatum* has been commonly employed as a crude drug in Indian traditional medicine for the treatment of diarrhoea as well as for use as an astringent, antihelminthic, asphrodisacs, laxative, diuretic, stomachic, cardiotonic and refrigerant.

Antibacterial, anti-inflammatory and anti-allergic activities of standardized *P. granatum* (pomegranate) rind extract containing 13% w/w ellagic acid were studied by *in vitro* method [10]. These antibacterial activities can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids etc. that are present in these plants [11].

MATERIALS AND METHODS:

Fruit collection & extraction: Ripened pomegranate fruits were collected from local places and, *daru* fruits from different neighbouring forests of Dehradun. White membrane was separated, dried under shade and stored into fine powder using electric blender. Solvent extract of the plants was prepared by taking 50 g of dried powder sample and extracted by Soxhlet distillation apparatus using ethanol, petroleum ether (PE) and distilled water (DW).

Isolation of bacteria: A total of 150 dental plaque samples were collected from Uttaranchal Dental College and Hospital, Dehradun and different dental clinics of Dehradun. The samples were collected ascetically and inoculated in nutrient broth for 24 hrs at 37^{o}C. Inoculated samples were streaked on nutrient agar and other selective media. All the isolates were identified as four bacterial species viz., *Lactobacillus, Proteus, Staphylococcus and Streptococcus* species.

Assay for Antibacterial Activity using Agar Well Diffusion Method: The inoculums were adjusted according to 0.5 McFarland standard which was prepared by adding 0.05ml of Barium chloride ($BaCl_2$) (1.17% $BaCl_2.2H_2O$) to 9.95ml of H_2SO_4 (1%) with constant stirring. The inoculums of test strains was adjusted to 1.5 x 10^8 CFU/ml equal to that of the 0.5 McFarland standard by adding sterile distilled water. 20 ml of Muller Hinton was poured into sterile petri plates and allowed to solidify completely. 100µl of inoculum (1.5 x 10^8 CFU/ml) was spreaded with the help of a sterilized spreader onto the entire surface of agar plate.

The antibacterial activity of solvent extracts was done by agar well diffusion method [12]. After the medium was solidified wells of 6mm were made in the plates with the help of a cork borer. 200µl of the extracts (500mg/ml) was introduced into the wells separately and the plates were incubated overnight at 37°C. The experiment was performed under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition (DIZ) around the well.

RESULTS AND DISCUSSION: The dental plaque samples were collected ascetically and were streaked on nutrient agar and other selective media. The isolates identified were four types of bacterial species viz., *Lactobacillus, Proteus, Staphylococcus and Streptococcus* species. The data of antibacterial activity obtained by using *daru* (**Table 1**) was compared with

pomegranate (**Table 2**) Ethanol (E), petroleum ether (PE) and distilled water (DW) extracts were evaluated against both Gram positive and Gram-negative bacteria isolated from dental plaque samples. The ethanol extract showed the highest antibacterial activity, followed by petroleum ether and water extract. All extract of the WM of pomegranate fruit were found to be most active against *Streptococcus sp.* and the least activity was shown against *Proteus sp.* In case of ethanol *daru* extract maximum zone of diameter was 27 mm by *Streptococcus sp.,* 26mm by *Lactobacillus & Staphylococcus sp.,* and 24 mm by *Proteus sp.* Lowest zone (18 mm) was shown by *Proteus sp.* against pomegranate DW extract.

TABLE 1: INHIBITORY ACTIVITY OF E, PE, DW EXTRACT OF *DARU*. (MAX. DIZ IN mm)

Test organism	E	PE	DW
Streptococcus sp.	27	26	24
Lactobacillus sp.	26	25	25
Staphylococcus sp.	26	24	23
Proteus sp.	24	22	19

TABLE 2: INHIBITORY ACTIVITY OF E, PE, DW EXTRACT OF *POMEGRANATE* (MAX. DIZ IN mm)

Test organism	E	PE	DW
Streptococcus sp.	26	25	23
Lactobacillus sp.	25	24	19
Staphylococcus sp.	24	24	20
Proteus sp.	23	22	18

There are many research carried out to investigate ethnobotanical uses of plants prevailing among native people [13]. Pomegranate is suitable alternatives and is now a subject of intense scientific study. The pomegranate fruit has valuable compounds in different parts of the fruit- peel, white membrane and arils. Another important product obtained from pomegranate fruit is the juice that can be obtained from arils or from whole fruit. Nowadays it is widely accepted that the beneficial health effects of fruits in the prevention of disease are due to the bioactive compounds they contain [14]. White membrane of pomegranate fruit contains high polyphenol concentration as compared to seeds. The edible part of the pomegranate fruit (50%) consists of 40% arils and 10% seeds. Edible part of fruit contain 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin, organic acid such as ascorbic acid, citric acid, and malic acid, and bioactive compounds such as phenolics and flavonoids, principally anthocyanins [15, 16].

Badria and Zidan [17] reported that pomegranate flavonoids have shown modest antibacterial action *in vitro* for strains relevant to gingivitis, although pomegranate flower extract can inhibit *in vitro* by both competitive and non-competitive mechanisms, a bacterial sucrose digesting enzyme responsible for initiating oral problems, including gingivitis [18]. Pomegranate rinsing also lowered saliva activities of α-glucosidase, a sucrose-degrading enzyme and increased activities of ceruloplasmin, an antioxidant enzyme [19]. The chemical composition of the fruits differs depending on the cultivar, growing region, climate, maturity, cultivation practice, and storage conditions [20, 21, 22]. Thus, it is very much necessary to analyze the potential of the plants in combating the antibiotic resistant organisms.

Although, mechanical and chemical plaque control methods have the potential to maintain adequate levels of oral hygiene, clinical experience and population-based studies have shown that such methods are not being employed as accurately as they should by a large number of people. Currently a wide range of options in oral antiseptics and toothpastes are available in the market which contain synthetic and/or natural compounds with antimicrobial activity [23]. Natural extract of *Punica granatum* is included in the formulation of commercially available oral hygiene products. The addition of natural extract aims to improve the antibacterial action, since these natural extracts have demonstrated effect against a wide range of microorganisms [24].

The ethanol extract has exhibited maximum antibacterial activity against tested bacterial strains when compared to other extracts used in the study. Our findings suggest that an appropriate bioactive compound(s) may be developed from white membrane as complementary alternative medicine for the treatment of dental infection causing bacterial strains.

CONCLUSION: Ethanolic *daru* extracts have great potential as antimicrobial compounds against dental bacteria. Thus, *daru* can be used in the treatment of dental diseases caused by bacteria. Medicinal plants

are considered as clinically effective and safer alternatives to the synthetic antibiotics.

Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

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