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Antibacterial Activity of *Punica granatum* in Different Solvents

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In this study, the antibacterial activity of leaf of *Punica granatum* was investigated. Different solvents used were, water, ethanol, methanol, acetone, propanol, 1,4-dioxan, N,N-dimethylformamide (DMF) and benzyl alcohol. The selection of solvents was on the basis of their polarity. The antibacterial activity of six clinical strains (*S. paratyphi, S. aureus, S. epidermidis, E. aerogenes P. aeruginosa and B. subtilis*) was determined by Growth inhibition using Agar ditch diffusion assay. The aqueous extract was able to inhibit only *B. subtilis* and *S. aureus* and was ineffective against all the other four bacterial strains. On the other hand organic solvents proved much better in inhibiting the studied bacterial strains except benzyl alcohol extract which was ineffective against

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all tested bacterial strains. Among the various solvents, 1,4-dioxan proved to be best while propanol was least effective. Finally, *E. aerogenes* was found to be most resistant bacteria, while *S. aureus, S. epidermidis* and *B. subtilis* were most susceptible.

Herbal medicine is the oldest form of healthcare known to mankind. Many drugs commonly used today are of herbal origin. Herbal medicine can be broadly classified into various basic systems: Traditional Chinese Herbalism, which is part of Traditional Oriental Medicine, Ayurvedic Herbalism, which is derived from Ayurveda, and Western Herbalism, which orlginally came from Greece and Rome to Europe and then spread to North and South America. Traditional medicine is an important source of potentially useful new compounds for development of chemotherapeutics.

Scientific research on medicinal plants should identify the active principles in the plants; scientific examination of the remedies could lead to standardization and quality control of the products to ensure their safety. It is after such evaluations that they can be approved for use in the primary health care. Such research activities could also lead to the development of new drugs as in the past[1,2].

Herbs are seed producing annual, biennial or perennial plants that do not develop a persistent woody tissue. Perhaps because herbs have such an important historical and tradition in healing, sometimes they are treated as a special category of plants i.e., those particularly valued for their medicinal, savory or aromatic qualities. An integrated health care system where resources of the traditional and orthodox medical systems are combined, as is being practiced in *Clinique de* Manongarivo in Madagascar, would be ideal especially for developing countries endowed with rich plant genetic resources[3].

In India, over 2600 plant species have been considered useful in the traditional system of medicines like Ayurveda, Unani, Siddha and Home Remedies[4]. A country like India is very much suited for development of drugs from medicinal plant. India has a rich heritage of knowledge on plant-based drugs both for use in preventive and curative medicines. One of the ancient classics, *Charak Samhita* is the oldest text available on the complete treatment of diseases, which specifies the use of hundreds of herbs in the complete treatment of bacterial diseases that include diarrhea, leprosy and tuberculosis[5]. There are over 50 000 botanically derived compounds with antimicrobial characteristics, but many of them are relatively weak and have narrow specificity. Several workers have reported the positive effects of the medicinal plants against number of strains of Gram positive as well Gram negative Bacteria[6].

Punica granatum is a shrub belonging to the unigeneric family Punicaceae, a native of semitropical Asia widely naturalized pan tropically. Earlier authors included the genus in the family Lythraceae[7]. The different parts commonly used are leaf, flower, fruit, fruit rind, seed, dried bark of stem and root. The leaf powder with sandal wood paste, curd and honey is sometimes used to check miscarriage[8]. Pericarp (rind) of the fruit is antidiarrhoeial and antidysentric and is used in combination with cloves[9]. The plant is also useful in snake and scorpion bite[10,11]. The root bark with milk is often used as a remedy in the enlargement of liver in children[12]. Some nonprotoplasmic cell contents like alkaloid, tannin, sugar, starch, fat, protein, mucilage, lignin, suberin, saponin and cutin are present in the leaf, which react positively with different concentrations of acids, alkalies, salts and dyes[13,14]. The chemical constituents of different parts of *Punica granatum* were reported earlier by different authors[15]. Horticultural form of *Punica granatum*, which does not yield fruits, is reported as ornamental. This type is popularly known as Gulnar, which blooms thrice a year during March and April, July and August and mid-January[16].

Considering the aforesaid, the objective of the present study was to evaluate the effect of *P. granatum* leaf on some bacterial strains that include *P. aeruginosa, S. paratyphi, S. aureus, S. epidermidis, E. aerogenes* and *B. subtilis*. The leaf extracts were prepared in water, ethanol, methanol, propanol, acetone, benzyl alcohol, 1,4-dioxan and N,N-dimethylformamide (DMF) and their antibacterial activity was evaluated.

The plant part selected for antibacterial study was the leaf of *P. granatum*. Fresh leaf material of *P. granatum* was collected randomly, in the month of August 2002 at Rajkot, Gujarat. They were washed thoroughly under running tap water for 2 h and then gently dried and 25 g was macerated with the help of a homogenizer. The slurry was taken in a conical flask and 100 ml of the solvent was added. It was then kept on a rotary shaker at 180-190 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was made to evaporate so as to make the

final volume one-fourth of the original volume. It was then stored in airtight bottles for further phytochemical and microbiological assays. The solvents were selected to represent a wide range of polarities and selectivity groups[17].

P. aeruginosa was isolated from urine. Skin infections, urinary infections, respiratory infections, external ear infections, septicaemia are caused by this strain. *S. aureus* has been isolated from sputum. Abscesses, boils, conjunctivitis, septicemia, endocarditis, osteomyelitis, pneumonia and empyema, mastitis, antibiotic associated enteritis, food poisoning, scalded skin syndrome in children, toxic shock syndrome are caused by this strain. *S. epidermidis* was isolated from pus. Bacteraemia is generally caused by this bacterium. *E. aerogenes* was isolated from stool. This opportunistic pathogen causes Urinary infections, bacteraemia, wound infections and hospital infections. *B. subtilis* has been isolated from sputum. Eye infections, endocarditis, meningitis are caused by this opportunistic pathogen. *S. paratyphi B* was isolated from faeces sample. Enteric fever (paratyphoid) is the disease caused by this bacterium. These bacterial strains were obtained from a private clinical microbiological laboratory (Micro Care Laboratory, Rajkot).

A loop full of the test strain was inoculated in 25 ml of nutrient broth and incubated for 24 h on a rotary shaker so as to activate the given test bacterial strain. The nutrient agar (IP) plates were prepared for the study of *in vitro* antibacterial activity by agar diffusion method (IP). Agar well diffusion method[18] was performed for the antibacterial assay.

Inoculation of the test strain was done by the Pour-plate technique. 0.2 ml of the activated strain was inoculated into the media when it reached 40-45° temperatures. The complete procedure of the plate preparation was done under laminar airflow to maintain strict sterile and aseptic condition. The solidification of the media took about 30 min. After the media got solidified, a ditch was made in the plates with the help of cup-borer (0.85 cm) and then it was filled with the test compound. The inhibitory activity of the compounds

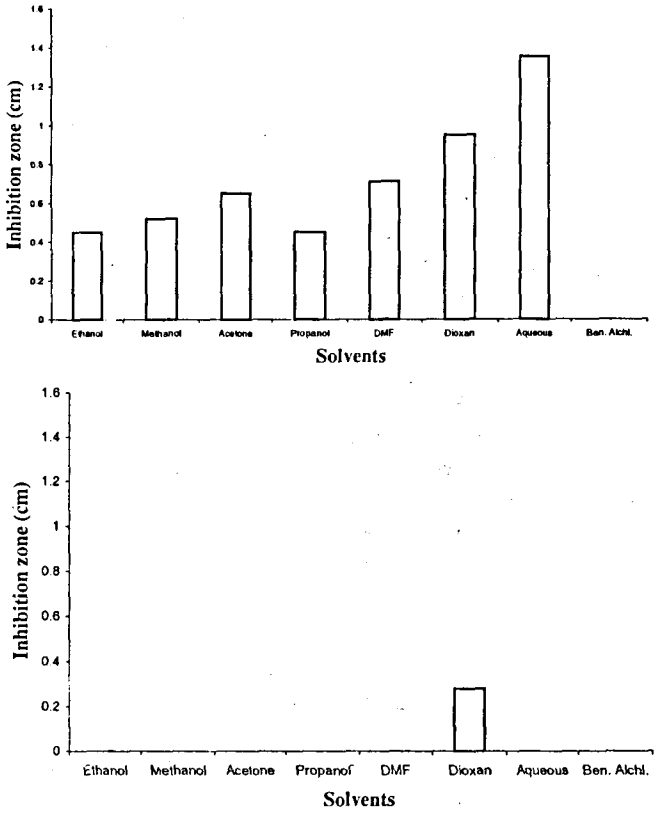

Fig. 1: Antibacterial activity of *P. granatum* against *B. subtilis* and *E. aerogenes*

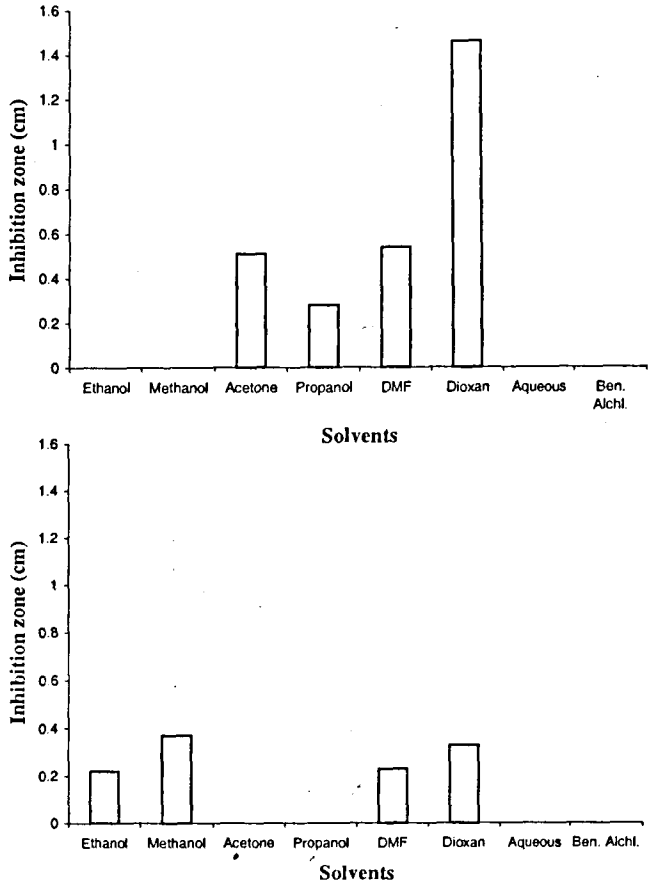

Fig. 2: Antibacterial activity of *P. granatum* against *P. aeruginosa* and *S. paratyphi* B

in various solvents was determined by comparing the sizes of inhibition zones of the different compounds in different solvents with those of the controls. The inhibitory zone of the control was subtracted from the test inhibitory zone and the same is plotted.

The effect of *P. granatum* extracted in different polar and non-polar solvents against Gram positive bacteria *B. subtilis* and Gram negative bacteria *E. aerogenes* is shown in fig.1. Maximum inhibition zone was produced by aqueous extract. The inhibition was about 1.4 cm. The non-polar solvent 1,4-dioxan was the next solvent to produce an inhibitory zone of 0.9 cm. It was followed by DMF, acetone, ethanol and methanol (0.4-0.5 cm). Propanol produced minimum inhibitory zone, while benzyl alcohol was not effective at all. These solvents produced an entirely different trend against *E. aerogenes*. It appears that this bacteria is highly resistant because except the compound extracted in 1,4-dioxan none of the compounds extracted produced any inhibition activity against this organism.

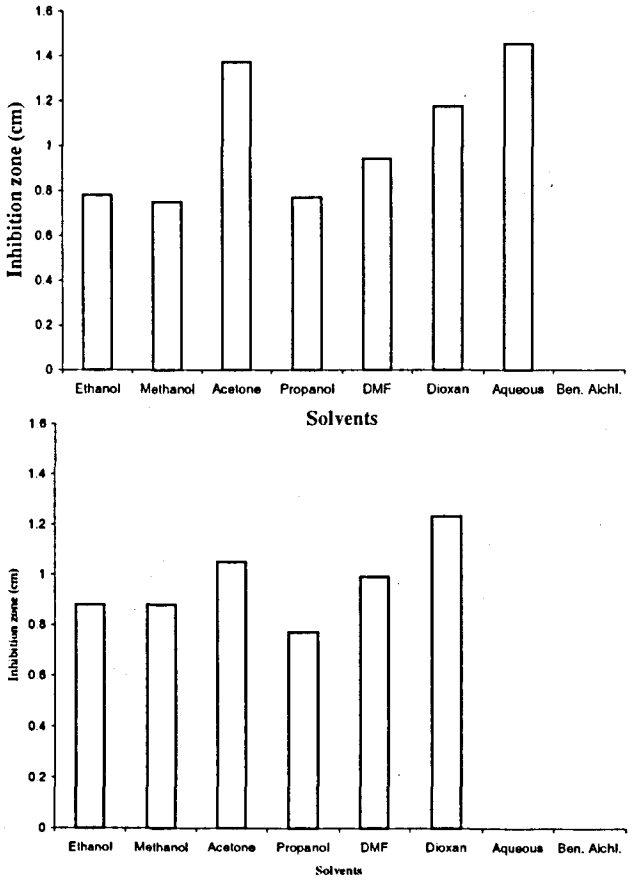

Fig. 3: Antibacterial activity of *P. granatum* against *S. aureus* and *S. epidermidis*

Fig. 2 shows the inhibitory activity of the compounds extracted in different solvents against Gram negative bacteria *S. paratyphi B* and *P. aeruginosa*. Both these bacterial strains were resistant towards compounds extracted in aqueous and benzyl alcohol solvents. DMF and 1,4-dioxan both solvents inhibited both the bacterial strains; both these solvents showed significant activity against *P. aeruginosa*, while moderate activity against *S. paratyphi B*. Amongst these two solvents 1,4-dioxan showed significant activity while DMF was moderately effective. When other organic solvents were considered ethanol and methanol inhibited *S. paratyphi B* but did not inhibited *P. aeruginosa*; while acetone and propanol showed opposite trend that is they could inhibit *P. aeruginosa* moderately but were not effective against *S. paratyphi B*.

P. granatum extracted in almost all the solvents showed significant activity against Gram positive bacteria *S. aureus* and *S. epidermidis* (fig. 3). Benzyl alcohol was ineffective against both these bacteria while aqueous extract showed significant activity against *S. aureus* but had no effect on *S. epidermidis*. All the solvents showed significant activity against *S. aureus* but maximum activity was in acetone followed by 1,4-dioxan. All the other solvents had almost equal activity. All the organic solvents had similar significant activity on *S. epidermidis*.

From the above results, it can be seen that most resistant bacteria was *E. aerogenes*, while most susceptible was *S. aureus, S. epidermidis* and *B. subtilis*; while S. *paratyphi B* and *P. aeruginosa* were moderate. Plant extracts are complex and isolating bioactive compounds is challenging. Only a few extractants have generally been used for isolating antimicrobial compounds from plants and Cowan[19] concluded that many classes of compounds are commonly obtained in only one solvent. In the present study, the organic solvents proved to be much better than aqueous solution. The aqueous extract was able to inhibit only two bacterial strains (*B. subtilis* and *S. aureus*) while it was ineffective against all the other four bacterial strains (*P. aeruginosa, E. aerogenes, S. paratyphi B, S. epidermidis*). Amongst the solvents studied 1,4-dioxan proved to be best while propanol was least effective. 1,4-dioxan showed significant activity on *B. subtilis, P. aeruginosa* and *S. aureus* and *S. epidermidis* while poor activity on *E. aerogenes* and *S. paratyphi B*. On the other hand propanol showed poor to moderate activity on all the bacterial strains.

The discriminatory effect of different solvents against different bacterial strains suggests the presence of different chemical compounds. The extracts of plants given as a whole might reduce the effect since each plant part has (perhaps) different compounds and their extractability in different solvent varies. Therefore it is necessary to screen the most active compound i.e. the best solvent for a particular compound and then it can be selected for further investigations to determine its therapeutic potential and may be for the synthesis of analogues with improved activity. The results of the present study suggest that the most effective is the compound extracted in 1,4-dioxan. The beneficial action of *P. granatum* might be due to a combination of phytochemicals acting collectively or synergistically.

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Physico-Chemical Aspects of Protein Binding of Nimesulide

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The binding of nimesulide, a cox-2 inhibitor, to bovine serum albumin was investigated by equilibrium dialysis method at different temperatures and pH conditions. The Scatchard plots were prepared based on these drug-protein binding data. The number of binding sites (n), the value of association constant (K) at different conditions and different thermodynamic parameters (i.e., standard free energy change $\triangle G^{\circ}$, standard enthalpy change $\triangle H^{\circ}$ and standard entropy change $\triangle S^{\circ}$) of nimesulide-BSA binding were determined. The result shows that number of binding sites

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