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PUNICA GRANATUM L. STEM AND LEAVES. A SOURCE OF ANTIMICROBIAL ACTIVITY

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ABSTRACT

Traditional medicine using herbal drugs exists in every part of the world. Although the philosophies of these traditional medicines have resemblance with each other, they differ widely from modern Western medicine. In view of the progress of western medicine, not only new synthetic drugs but also herbal drugs have to fulfill the international requirements on quality, safety and efficacy. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine. The plant *Punica granatum* is commonly called as pomegranate and is used for the present study. The test organisms used were *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus & Streptomyces pyogenes* to check the antimicrobial activity of *Punica granatum.* The observations have shown that the antimicrobial activity is present in all the five test organisms and in the increasing concentration of the plant extract suggesting that this plant can possibly be used as medicine as an antimicrobial agent.

KEYWORDS: *Punica granatum, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Streptomyces pyogenes,* antimicrobial activity.

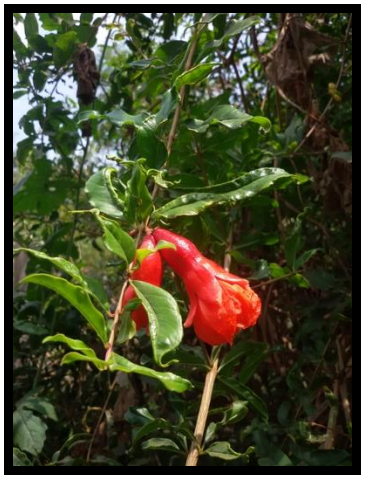
INTRODUCTION

The plant *Punica granatum* belongs to family Lythraceae and is commonly called as pomegranate (Almeida, 1990). The term Pomegranate is derived from Medieval Latin word "*pomum*" means "apple" and "*granatum*" means "seeded". Previously placed in its own family Punicaceae but recent phylogenetic studies have shown that it belongs to the family

Lythraceae according to APG system. The genus having two species *Punica granatum* L. and *Punica protopunica* Balf. The term "*Punica*" is derived from Latin word which means "pertaining to Carthage – Cartheginian apple" and *"granatum*" means "filled with seeds". The plant is deciduous small tree, branches sometime spiny, leaves are simple, oblong or obovate, axillary or terminal inflorescence, actinomorphic, bisexual red flowers having gamosepalous and polypetalous condition, numerous stamens, inferior ovary with axile and parietal placentation.

The various extracts (aqueous, ethanol, chloroform) of peel, whole fruit and seeds of *Punica granatum* have revealed the presence of triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins, carbohydrates and vitamin C (Bhandary *et al*, 2012).

Compounds belonging to the terpenoids, alkaloids and flavonoids are used as drugs or as dietary supplements to heal or prevent various diseases and some of these compounds seem to be competent in preventing and inhibiting various types of cancer (Kumar A. *et al*, 2013).



Punica granatum plant

USES

Pomegranate leaves are used to calm the stomach disorder or diarrhea triggered due to any kind of digestive problems. Drinking tea made from the leaves of pomegranate helps in curing digestive problems (Bhowmik *et al*, 2013). Bark of plant is anthelmintic and expelling tapeworm. Fruit juice is used in leprosy. The flowers yield red dye which is used for dyeing cloth (Bhattacharjee, 2008).

MATERIAL AND METHODS

The plant material i.e. leaves and stems of *Punica granatum* for the present work was collected from Pawan Baug Malad (W.) & authenticated.

The test organisms were procured from NCIM- *E. coli* NCIM 2066, *Pseudomonas aeruginosa* NCIM 2659, *Bacillus subtilis* NCIM 2724, *Staphylococcus aureus* NCIM 2121, *S. pyogenes* NCIM 2608.

METHOD: For antimicrobial activity Agar well diffusion assay (zone inhibition assay) was used. This is the most widely used method for determining the susceptibility of organisms to antimicrobials. In case of plant extract screening this method is widely applicable because turbidometry is very difficult to do when the extracts impart color to the broth.

In the molten Mueller Hinton agar around 0.2 ml (OD 0.04) of test strains were inoculated, Care was taken to ensure proper homogenization. The media was poured in sterile petriplates. The extracts obtained from the plants were used for studying their antimicrobial activity. A loop full of bacterial strain was inoculated in 30 ml of Nutrient broth in a conical flask and incubated for 24 hrs for bacterial culture at 37^{o}C and 48hrs for fungal cultures at room temperature (RT) to get active strain. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (7 mm). The extract compound (100 µl) was introduced into the well and plates were incubated at 37°C for 24 hrs for bacterial cultures. All samples were tested in triplicates. The plates were kept for pre-diffusion before keeping them for incubation. A control with DMSO was kept for all test strains as it was the diluent used for reconstituting the extracts so as to ascertain that it is not responsible for the activity demonstrated if any. The plates were observed for the presence of inhibition of bacterial growth that was indicated by the clear zone around the wells. The size of the zone of inhibition was measured and the antibacterial activity was expressed in terms of the average diameter of zone inhibition in millimeters. The absence of zone inhibition was interpreted as the absence of activity. The cups and concentration of extract in the cups is as shown in the fig 1.

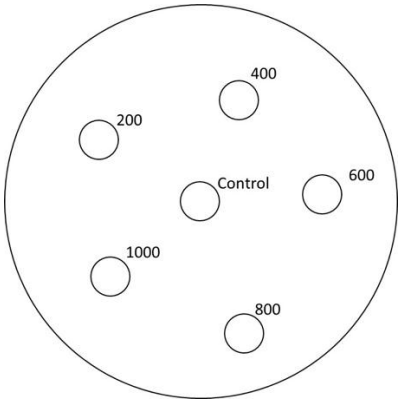
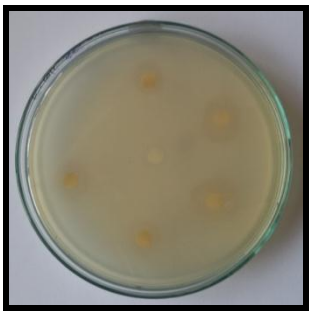


Fig: 1

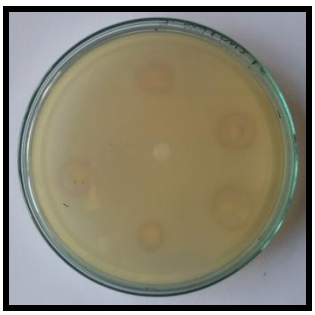
OBSERVATIONS

Name of Microorganism	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml	Ctrl DMSO
Pseudomonas aeruginosa	9	10	10	11	13	-
Staphylococcus aureus	9	10	11	11	13	-
Escherichia coli	8	9	10	11	12	-
Bacillus subtilis	11	12	12	13	14	-
Streptococcus pyogenes	10	10	11	11	12	-

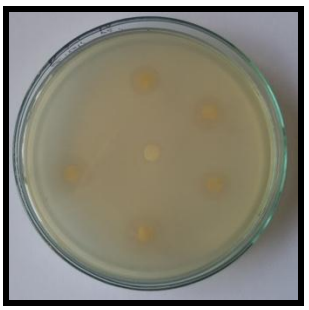
-- "Indicates No growth" / Zone of inhibition in mm. All values in this table represent the mean ±SD (n=3).



Pseudomonas aeruginosa

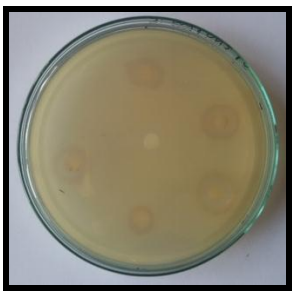


Staphylococcus aureus



Escherichia coli

Bacillus subtilis



Streptococcus pyogenes

CONCLUSION

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities. The local people have a long history of traditional plant usage for medicinal purposes. The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 4000 - 5000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rigveda, which is said to be written between 1600 - 3500 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians (an indigenous system of medicine) which are a basic foundation of ancient medical science in India (De Pasquale, 1984). The development procedure of herbal drugs for worldwide use has to be different from that of synthetic drugs (Vogel, 1991). Sumathi and Gowthami (2014) carried out phytochemical analysis and *in- vitro* antimicrobial activity of aqueous and solvent extracts of *Carica papaya* against clinical pathogens: *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhii, Staphylococcus aeures, Candida albicans.* Anzana Parvin et. al. (2013) carried out *in vitro* to determination of antibacterial activity of *Polyalthia longiflia* (Debdaru) leaf extracts with hexane, methanol and chloroform against six tested pathogenic bacteria viz. *Bacillus subtilis, Sarcina lutea, Xanthomonas compestris, Escherichia coli, Klebsiella pneumonia and Pseudomonas* sp. The results obtained were significant hence plant leaf extract can be used in the treatment of infectious diseases caused by them. Antimicrobial activity of marketed samples of *Holarrhena antidysenterica* by Vaidya (2015), of aqueous and methanolic extracts of young and mature leaves of *Psidium guajava* has been studied by Vaidya (2013) and of *Eclipta prostrata* has been reported by Vaidya and Sambhare (2016). Pharmacognostic studies and fluorescence analysis of *Punica granatum* have already been studied by Vaidya and Roy (2019 a and b). Vaidya (2015) has also studied the stomata of some members of *Philodendron* of family Araceae.

From the observations it is seen that all the test organisms show zone of inhibition. The maximum zone of inhibition is against *B. subtilis* > *S. aureus* > *P. aeruginosa* > *S. pyogenes* > *E. coli* and in the increasing concentration of the plant extract. Thus the plant *P. granatum* stem and leaves can possibly be used as an antimicrobial agent.

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