Phytochemical screening and antibacterial activity of aqueous leaf extract of *Punica granatum*

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Abstract

In the present study the leaves of *Punica granatum* were subjected to phytochemical screening and the impact of the leaf extract of *Punica granatum* was tested against *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi*. The phytochemical analysis revealed the presence of various phytochemicals such as alkaloids, tannins, phenols, flavonoid and saponins.

Agar disk diffusion method was employed to test the antibacterial activity and the result showed that the aquesous leaf extract of *Punica granatum* showed 100% inhibition against all the tested bacteria at varying concentration of extract. The Minimum Inhbitory Concentration (MIC) of *Punica granatum* aqueous leaf extract that showed 100% inhibition was 0.36 mg/ml, 0.13 mg/ml and 0.13 mg/ml in case of *Bacullus subtilis*, *Staphylococcus aureus* and *Salmonella typhi* respectively.

Key words: Punica granatum, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis, MIC.

Introduction

Natural products are the source of synthetic and traditional medicine. Phytochemicals are compounds occurring naturally in plants. The use of plants and plant products as medicines could be traced as far back as beginning of human civilization. Plant extracts and compounds derived from plants are in use as drug from the ancient times **[1].** The Indian subcontinent has a rich flora of various plants used in traditional medical treatments **[2]**.

These plants contain different bioactive ingredients used to cure disease or relieve pain **[3].**

Punica granatum L., commonly known as pomegranate, is a fruit bearing deciduous shrub or small tree, native to Asia and belongs to family Lathraceae (Altuner, 2011) **[4]**. the leaves are shiny and about 7.6 cm long **[5]**. Different parts of plants such as leaves, bark, and fruit have medicinal significance **[6]**. *Punica granatum* has been used as traditional medicine in many countries for the treatment of dysentery, diarrhoea, helminthiasis, acidosis, hemorrhage etc. **[7].** Numerous phytochemical constituents have been reported to be present in different parts of the *Punica granatum* plant, which makes it medicinally important.

Owing to the above apprehensions the present study was undertaken to screen the phytochemical constituents and antimicrobial activity of aqueous leaf extract of *Punica granatum*.

Materials and Methods

PLANT MATERIALS: the fresh tender leaves of *Punica granatum* was collected. The leaves were washed with deionised water and disinfected with 0.1% $HgCl_2$ solution for 5 min and dried in shade away from light for 15 days and ground to fine powder using electrical grinder and sieved **[8,9]**

PREPARATION OF PLANT EXTRACT: the fine powder of *Punica granatum* was made into thimble for loading in Soxhlet apparatus and extraction was done continuously for 72 hours. The extract thus obtained was concentrated under vacuum rotary evaporator and extracts were kept in desiccators until used **[10, 11]**

PHYTOCHEMICAL SCREENING: phytochemical screening

were conducted in *Punica granatum* leaf extract according to previous published standards **[12, 13]**

ANTIBACTERIAL ANALYSIS: The organisms namely

- *Staphylococcus aureus*
- *Bacillus subtilis*
- *Salmonella typhi*

Were used during the present experiment and were procured from Hi-media. These organisms are potential causative agents for different diseases.

AGAR DISC DIFFUSION METHOD: Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 ° C for 48 hrs. The agar plates of the above media were prepared and wells were made in plate each plate was inoculated with 18h hold cultures (100µ, 104 CFU and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of samples. The control wells were filled with Gentamycin along with solvent. All the plates were incubated at 37°C for 24 h and the diameter of Zone of Inhibition (ZOI) were noted **[14]**.

MEDIA USED: peptone – 10g, NaCl – 10g and Yeast extract 5g, agar – 20g in 1000 ml of distilled water.

Results and Discussion

PHYTOCHEMICAL SCREENING: the results of phytochemical screening is presented in Figure 1.. The results showed the presence of various phytochemicals such as alkaloid, flavonoid, saponin, tannin and phenol. The phytochemicals revealed during the phytochemical screening showed lowest concentration of alkaloid (3.07 ± 1.0 mg/ml) and highest content of flavonoids (81.16 ± 0.36 mg/ml). According to Kumar *et al.* (2015) **[10]** the medicinal properties of the plants are due to the presence of the secondary metabolites (alkaloids, phenols etc.) in different parts. The phenols posses redox properties and thus impart antioxidant properties to the plants in which they are present **[10].** Tannins, alkaloids, saponins and flavonoids have been found to be active antibacterial agents **[15].** Thus taking into consideration the phytochemicals present in the leaf extract, *Punica granatum* leaf extract appears to be an effective potent antimicrobial agent.

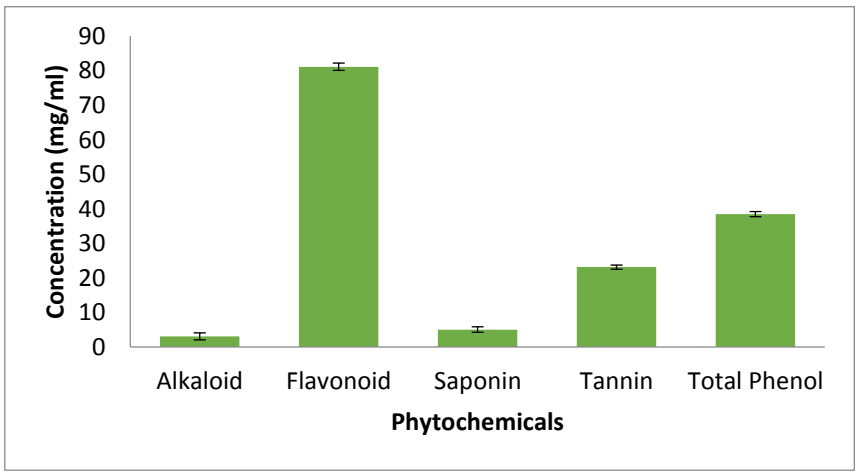

Figure 1: Results of Phytochemical screening of aqueous leaf extract of *Punica granatum*

ANTIBACTERIAL ANALYSIS: The antibacterial activity of *Punica granatum* aqueous leaf extract against *Staphylococcus aureus, Bacillus subtilis* and *Salmonella typhi* was determined using the agar disk diccusion method. The results of antibacterial screening, the agar plates showing the zone of inhibition (ZOI) of aqueous leaf extract of *Punica granatum* is presented in figure 2, and the numerical value of the ZOI is presented as table 1.

Table 1: Showing Zone of inhibition (ZOI) in mm and Minimum inhibitory concentration (MIC) against tested microbes

Bacteria	Concentration of extract (mg/ml)			MIC (mg/ml)
	0.13 mg/ml	0.36 mg/ml	0.612 mg/ml	
Bacillus subtilis	ND	3	5	0.36 mg
Staphylococcus aureus	2.5	6	10	0.13 mg
Salmonella typhi	4.6	8.0	14.0	0.13 mg

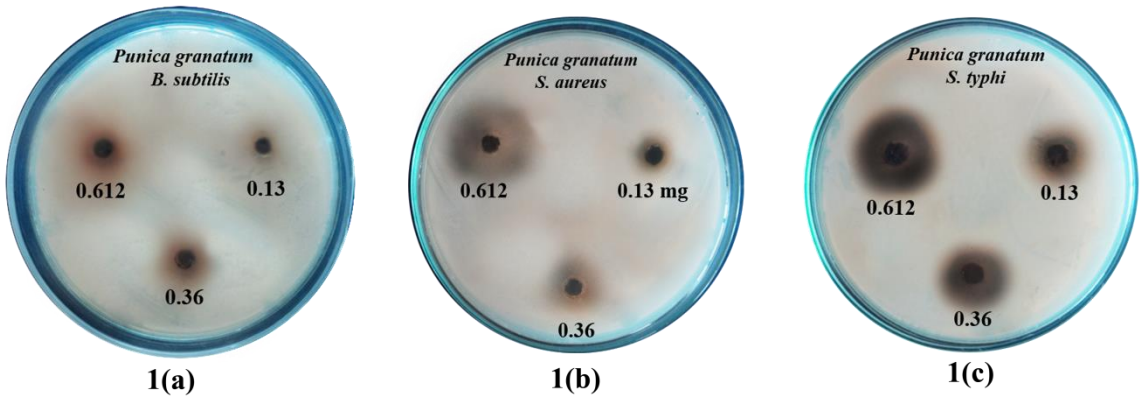

Figure 2: Agar plates showing the Zone of inhibition (ZOI) of aqueous leaf extract of *Punica granatum* against tested microbes

Several phytochemicals have been known to possess antimicrobial properties. Tannins, alkaloids, saponins, flavonoids and sterols have been found to inhibit the growth of *Salmonella typhi* **[14].** Middleton and Kandaswami (1994) **[17]** showed that flavonoids inhibit several enzymes, chelate certain metal cation, affects protein phorphorylation. Smith (1996) **[18]**concluded that flavonoids interpose several membrane linked processes. Tannins form irreversible complexes with prolene rich protein resulting in inhibition of cell synthesis in bacteria **[11, 16].** The leaf extract of *Punica granatum* was found effective against all the test bacteria. The MIC (minimum inhibitory concentration; the concentration of plant leaf extract showing 100 % inhibition) of *Punica granatum* aqueous leaf extract in case of *B. subtilis, S. aureus* and *S. typhi* were found to be 0.36 mg/ml, 0.13 mg/ml and 0.13 mg/ml respectively. *S. aureus* is a gram +ve bacteria found frequently in human respiratory tract and on the skin. It is common cause of skin infections (eg. Boils), respiratory diseases (eg. Sunusitis), and food poisoning. *B. subtilis* is a gram +ve bacteria, found in gastrointestinal tract of ruminants and humans. It is also known to cause diseases in several immunocompromised patients. *S. typhi* causes typhoid fever, paratyphoid fever in humans.

The obtained results showed that the aqueous leaf extract of *Punica granatum* was effective as and antibacterial agent against tested bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhi*). The demonstration of antibacterial activity of aqueous leaf extract of *Punica granatum* is indeed a promising development, which will help to discover new chemical classes (Medicines).

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