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# Antibacterial activity for fruit peel Methanol extract of *Punica granatum* Linn. (Punicaceae)

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**Abstract:** Antibacterial activity of the methanol extract of fruit peel of *Punica granatum* Linn (Family: Punicaceae) was evaluated against two Gram positive and two Gram negative bacteria. The Gram positive bacteria included *Staphylococcus aureus, Streptococcus pneumoniae* and the Gram negative organisms included *Escherichia coli* and *Pseudomonas aeruginosa* respectively. The culture media used for antibacterial assay was Mueller Hinton agar for the growth of *S. aureus, E. coli* and *P. aeruginosa.* The media used for the growth of *S. pneumoniae* was Mueller Hinton blood agar. The antibacterial assay was performed through Disc diffusion technique. The methanol extract was tested at three different concentrations (50, 100 and 200 mg/ml). Standard antibiotic discs containing vancomycin (30 µg) for *S. pneumoniae,* penicillin (10 units) for *S. aureus,* ceftriaxone (30 µg) for *E. coli* and ciprofloxacin (5 µg) for *P. aeruginosa* were used for the activity comparison. The results of the study revealed that the extract possesses antibacterial activity against *S. aureus*, *S. pneumoniae* and *P. aeruginosa* at all tested concentrations. The maximum zone of inhibition of 19 mm of the extract at 200 mg/ml was observed against *S. pneumoniae.* However, no zone of inhibition was observed against *E. coli* at the tested concentrations of the extract. Based on the results obtained in this study, it may be concluded that the fruit peel of *P. granatum* possess broad spectrum of antibacterial activity against a number bacteria.
**Keywords:** *Punica granatum* Linn., Methanol extract, Antibacterial, Zone of inhibition.

## Introduction

A major cause of morbidity and mortality in recent years is believed to be result of manifestations of pathogenic bacteria. Even though a large number of new antibacterials have been discovered in recent years, resistance to these drugs still remains a global concern. Therefore, there is a pressing need to discover new antimicrobial agents. Among the potential sources for antibacterial compounds, plants have long been investigated. Owing to their low toxicity, there has been long tradition of using dietary plants to treat infections in various traditional and folk medicines across the globe (Djeussi et al., 2013). The knowledge and usage of herbal medicine for the treatment of various ailments among various tribes of Malaysia is still a major part of their life and culture. It was learnt that the tribes inherit rich traditional knowledge about the medicinal uses of flora and apply this knowledge for making crude phytomedicines to cure number of bacterial diseases and ailments since time immemorial.

*Punica granatum* Linn. (Fam. Punicaceae), commonly known as *'Delima'* is a large deciduous shrub or small tree native to to central Asia, but since the pomegranate tree is highly adaptive to a wide range of climates and soil conditions, it is grown in many different geographical regions. Recent scientific findings corroborate traditional usage of the pomegranate as a medical remedy and indicate that pomegranate tissues of

the fruit, flowers, bark, and leaves contain bioactive phytochemicals that are antimicrobial, reduce blood pressure, and act against serious diseases such as diabetes and cancer. These findings have led to a higher awareness of the public to the benefits of the pomegranate fruit, particularly in the western world, and consequently to a prominent increase in the consumption of its fruit and juice (Holland et al., 2009). The paste of fruit peel is applied to the affected part and is used as a remedy for treating burn wound (Siang, 1983). The local tribes apply the paste of ground peel over fresh cuts and wounds for quick healing and claim for its promising activity. The fruit peel is also believed to be useful in leprosy. The ground peel is applied to the affected part (Singh et al., 1980). A thorough literature survey from all available scientific sources revealed antiviral activity of the fruit peel against genital herpes virus (HSV-2). Voravuthikunchai *et al*., reported the antibacterial activity of the aqueous extract of dried aerial parts against *E. coli*. However, no reports on the antibacterial activity of the fruit peel were available in the literature. Consequently, in this paper, we have focussed on the antibacterial activity of the methanol extract of fruit peel of *P. granatum* Linn. on few selected bacterial species.

## Materials and Methods

### Collection and preparation of plant material

Fresh fruits of *P. granatum* were purchased from local market, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The peels were taken out, cut in to small pieces and then dried under shade. The shade dried plant material was pulverized in a mechanical grinder to obtain coarse powder.

### Preparation of extract

The dried powdered plant material (50 g) was extracted with methanol by maceration process at room temperature for 7 days. Following extraction, the marc was separated by filtration and the liquid extract was concentrated to yield dry residue (yield: 7.3%w/w with respect to dried plant material). The dried methanol extract was dissolved in DMSO in such a way that the final concentration of the extract would be 1g/ml of DMSO.

### Determination of antibacterial activity

### Test organisms

The antibacterial activity of the methanol extract was performed against two Gram positive and two Gram negative bacteria. The Gram positive bacteria included *Staphylococcus aureus, Streptococcus pneumoniae* and the Gram negative organisms included *Escherichia coli* and *Pseudomonas aeruginosa* respectively.

### Test media

The culture media used for antibacterial assay was Mueller Hinton agar for the growth of *S. aureus, E. coli* and *P. aeruginosa.* The media used for the growth of *S. pneumoniae* was Mueller Hinton blood agar. The pre-sterilized and sealed media supplied by M/s Fisher Scientific Sdn Bhd, Selangor was used in the study.

### Antibacterial assay

The invitro antibacterial activity of the methanol extract was carried out by disc diffusion method (Khalid *et al.,* 2011). Actively growing log phase cultures were streaked on to sterile agar plates containing sterile culture medium. Ready-made sterilized discs of size 6 mm were used, each having maximum capacity of 30 µl. The extract at concentrations of 50, 100 and 200 mg/ml was loaded on the discs.. The standard antibiotic discs contained vancomycin (30 µg) for *S. pneumoniae,* penicillin (10 units) for *S. aureus,* ceftriaxone (30 µg) for *E. coli* and ciprofloxacin (5 µg) for *P. aeruginosa* respectively. The filter paper discs were then placed on the medium containing the cultures and incubated for 24h at $37^0$C. The diameter of zone of growth inhibition was recorded. DMSO alone served as negative control.

## Results and Discussion

The results of the study (Table 1) revealed that the methanol extract of *P. granatum* fruit peel showed antibacterial activity against *S. aureus*, *S. pneumoniae* and *P. aeruginosa* at all tested concentrations.

**Table 1: Antibacterial activities of the methanol extract of *P. granatum* in disc diffusion assay**

| Test sample | Concentration | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | *S. aureus* | *S. pneumoniae* | *E. coli* | *P. aeruginosa* |
| **Methanol extract** | 50 mg/ml | 11.0 | 12.0 | - | 7.0 |
| | 100 mg/ml | 12.0 | 16.0 | - | 12.0 |
| | 200 mg/ml | 16.0 | 19.0 | - | 16.0 |
| **Penicillin** | 10 units/disc | 26.0 | - | - | - |
| **Vancomycin** | 30 µg/disc | - | 21.0 | - | - |
| **Ceftriaxone** | 30 µg/disc | - | - | 28.0 | - |
| **Ciprofloxacin** | 5 µg/disc | - | - | - | 30.0 |

**Note: The control disc used for solvent had no zone of inhibition, so this data is omitted from the above data. Inhibition zones including the diameter of the paper disc (6 mm).**

The activity was observed in a concentration dependant manner. The zones of inhibition of the extract for *S. aureus* were 11, 12 and 16 mm for 50, 100 and 200 mg/ml concentration respectively as against standard drug penicillin that showed 26 mm at 10 units/disc concentration. Similarly, the zones of inhibition of the extract for *S. pneumoniae* were 12, 16 and 19 mm for 50, 100 and 200 mg/ml concentration respectively (standard drug vancomycin showed 21 mm at 30 µg/disc concentration). For *P. aeruginosa*, the zones of inhibition of the extract were 7, 12 and 16 mm for 50, 100 and 200 mg/ml concentration respectively (standard drug ciprofloxacin showed 30 mm at 5 µg/disc concentration). The study revealed maximum zone of inhibition against *S. pneumoniae.* However, no zone of inhibition for the extract was observed against *E. coli* at the tested concentrations.

## Conclusion

The spectrum of activity observed in the present study may be an indicative of the presence of broad spectrum antibacterial compounds in the extract since the extract was found to be active against both Gram positive and Gram negative bacteria. Further studies are recommended to screen the methanol extract over a wide range of other microorganisms to determine its spectrum of activity. It would also be interesting to isolate possible phytoconstituents responsible for the said effect.

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