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## Evaluation of the Antibacterial Activity in Pomegranate Peels and Arils by using Ethanolic Extract against *S. Mutans* and *L. Acidophilus*

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**Abstract-** The use of antibiotics has revolutionized the treatment of various enteric bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms, thus necessitating the need for development of novel antimicrobials. The main objective of this study is to evaluate antibacterial activity of pomegranate fruit extract on selected bacterial culture. Antibacterial activity of pomegranate was tested on MRS agar plates by employing punch well technique. Various concentrations of the peels, arils and peels and arils mixture (1:1) prepared by dissolving in Dimethyl Sulphoxide to obtain a final concentration of 10g/ml, 5g/ml, 2.5g/ml and 1.25g/ml against the test organisms. The sensitivity of bacterial strains to aqueous and alcoholic extracts of the peels and arils of *Punica granatum* calculated by measuring the diameter of inhibition zone.

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**GJMR-J Classification:** NLMC Code: QV 50, W 100



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# Evaluation of the Antibacterial Activity in Pomegranate Peels and Arils by using Ethanolic Extract against *S. Mutans* and *L. Acidophilus*

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**Abstract-** The use of antibiotics has revolutionized the treatment of various enteric bacterial infections. However, their indiscriminate use has led to an alarming increase in antibiotic resistance among microorganisms, thus necessitating the need for development of novel antimicrobials. Then main objective of this study is to evaluate antibacterial activity of pomegranate fruit extract on selected bacterial culture. Antibacterial activity of pomegranate was tested on MRS agar plates by employing punch well technique. Various concentrations of the peels, arils and peels and arils mixture (1:1) prepared by dissolving in Dimethyl Sulphoxide to obtain a final concentration of 10g.ml, 5g.ml, 2.5g.ml and 1.25g.ml against the test organisms. The sensitivity of bacterial strains to aqueous and alcoholic extracts of the peels and arils of *Punica granatum* calculated by measuring the diameter of inhibition zone. Result showed combination of peels and arils extract has greater inhibitory effect. Arils have no inhibitory effect against selected organisms. Result showed combination of peels and arils have greater antibacterial effect than pure peel extract. Also result showed combination of peels and arils have greater antibacterial effect on *L. acidophilus* in comparison with pure peel extract. Also result showed pure peel extract has greater antibacterial effect on *S. mutans* in comparison with combination of peel and arils extract.

**Keywords:** pomegranate (*punica granatum*) peels, arils, *S. mutans*, *L. acidophilus*.

## I. INTRODUCTION

*Punica granatum* is one of the oldest known edible fruits. It has been widely used in traditional medicine worldwide for the treatment of different types of diseases (Olapour et al., 2010). Also several antioxidant activities, including radical-scavenging ability, ferrous ion chelating and ferric ion reducing antioxidant power, were identified on *P. granatum*.

Research showed low concentration of *P. granatum* extract led to delay in *S.aureus* growth, while in a higher concentration of *P.granatum* extract, growth of *S.aureus* was eliminated (Braga et al., 2005). *P. granatum* also has antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus* and *Klebsiella* (Fawole et al., 2012). Investigation on the chemical composition of pomegranate fruit led to identification of cyanidin-3-glucose, quercetin, gallic acid, pelargonidin-3-galactose and myricetin which has antibacterial activity

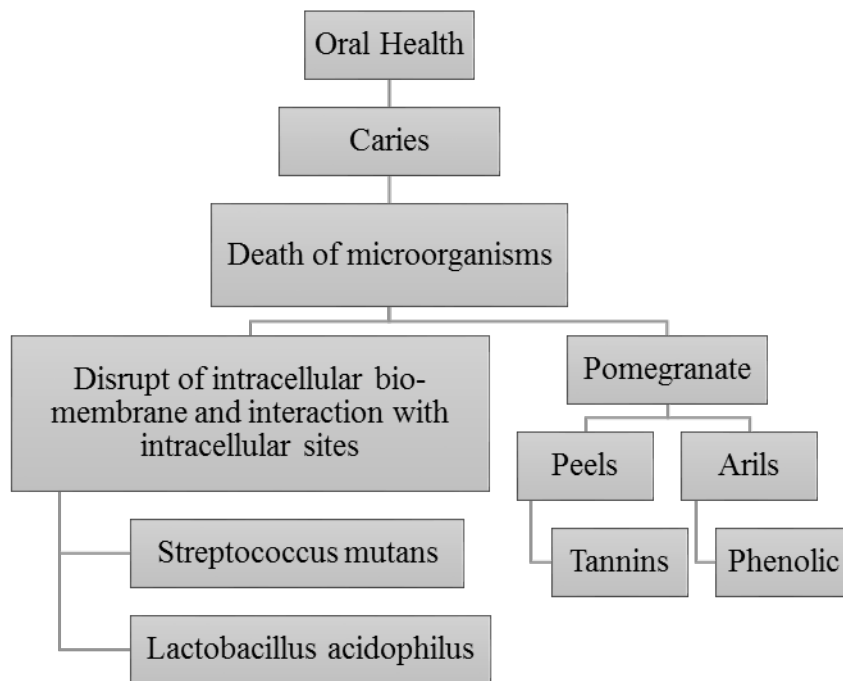
against *E. coli* and *B. subtilis*. Also research has shown that gallic acid has the highest antibacterial activity against *E. coli* and *B. subtilis* and the antibacterial activity of the compounds was due to the structural similarities of the compounds (Naz et al., 2007).

Although studies shows *Punica granatum* has antibacterial potential against few bacterial strains but there is lack of investigation on antibacterial property of *Punica granatum* against oral bacterial. Also the indiscriminate use of antibiotics led to an increase in antibiotic resistance between different microorganisms. This situation shows the need for development of novel antibiotics (Das et al., 2010).

Streptococcus mutans is the main microbial factor in dental caries and colonization of these bacteria in children is associated with dental caries (Lehl et al., 1999). Distribution of dental caries can be effectively reduced by reducing the carbohydrate in the diet and also result shown the number of oral lactobacilli has correlation with the amount of carbohydrate in the diet (Jay et al., 1938).

The aim of the study is to compare and measure antimicrobial effect of arils and peels extract of pomegranate between *S. mutans* and *L. acidophilus* which are main microbial factor in dental caries.

Conceptual or Theoretical Framework



## II. MATERIALS AND METHODS

### a) List of Materials

1. *Streptococcus mutans* and *Lactobacillus acidophilus*
2. Pomegranate
3. 95% Ethanol
4. MRS Agar
5. Dimethyl Sulphoxide (DMSO)
6. Disk paper
7. Whatman Filter paper
8. Micropipette
9. Micropipette tips
10. Incubator
11. Autoclave
12. Laminar Hood

### b) Preparation of Bacterial strain

Bacterial strains purchase from national institute of molecular biology and biotechnology (BIOTECH) University of the Philippines Los Baños, Laguna, Philippines.

### c) Methods of Extraction

Fresh pomegranate arils and peels were cleaned and separated. The peels and arils separately grounded blender. Fifty grams of blended peels or arils placed in 250ml Erlenmeyer flasks, followed by adding 100 ml of 95% ethanol. The flasks then shacked at room temperature for 18 h prior to filtration with Whatman paper. The filtrated mixtures were concentrated under reduced pressure using rotary evaporator at 40 °C. These crude extracts were kept at 4 °C until use.

### d) Measurement of Antibacterial Activity

Antibacterial activity tested on MRS agar plates by employing Punch well method. Various concentrations of the peel, arils and peel and arils mixture (1:1) prepared by dissolving in Dimethyl Sulphoxide (DMSO) to obtain a final concentration of 10g.ml, 5g.ml, 2.5g.ml and 1.25g.ml against the test organisms. The test inoculums swabbed uniformly onto the MRS agar plates and wells of diameter 8mm were punched out in each plate. 30µl of each of these extracts were pipetted out into these wells, the plates incubated upright at 37°C overnight. Dimethyl sulfate used as negative control. The sensitivity of bacterial strains to aqueous and alcoholic extracts of the different extract of *Punica granatum* calculated by measuring the diameter of inhibition zone. Bacteria showing a clear zone of inhibition >4mm considered to be sensitive. Experiments performed in triplicates for each combination of extract and the bacterial strain.

### e) Statistical Analysis

Result from experiment subjected to statistical ANOVA test. P-values < 0.05 considered as statistically significant. Graphs prepared using MS Excel 2010.

## III. RESULTS AND ANALYSIS

Result of ANOVA analysis showed there is significant difference between different concentration of different extract (P<0.01). Also result showed there is significant difference on inhibition of *S. mutans* and *L. acidophilus* in treated with different extract with different concentration (p<0.01)

Result showed combination of peels and arils extract has greater inhibitory effect. Result showed Arils

has no inhibitory effect against selected organisms. But result showed peels have inhibitory effect.

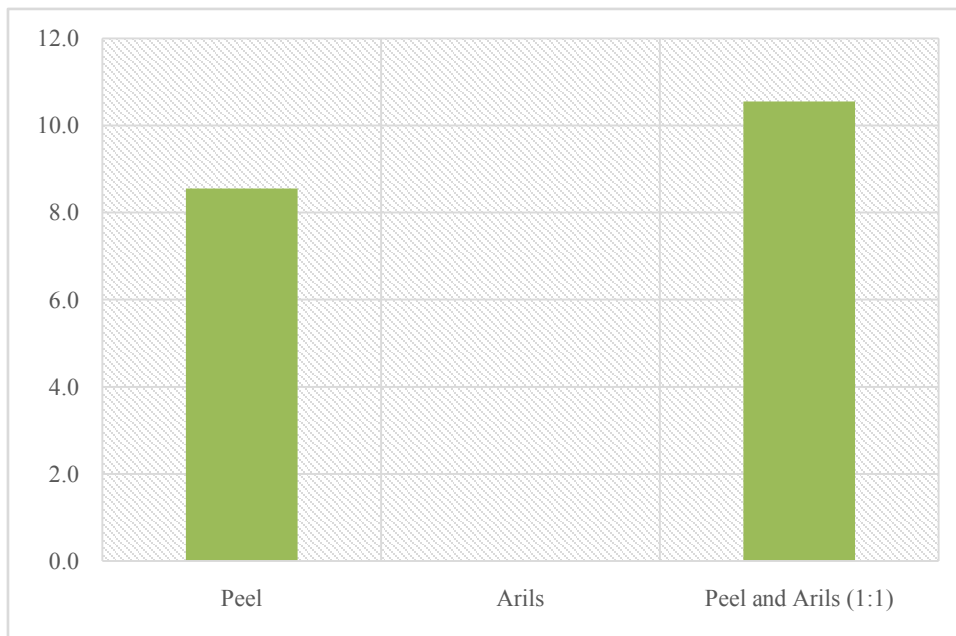


Figure 1 : Antibacterial effect of different extracts

Result showed by decrease in concentration of peel extract antibacterial effect of peel was decreased

(Figure 2) and *S. mutans* is more sensitive to peel extract than *L. acidophilus* (Figure 3).

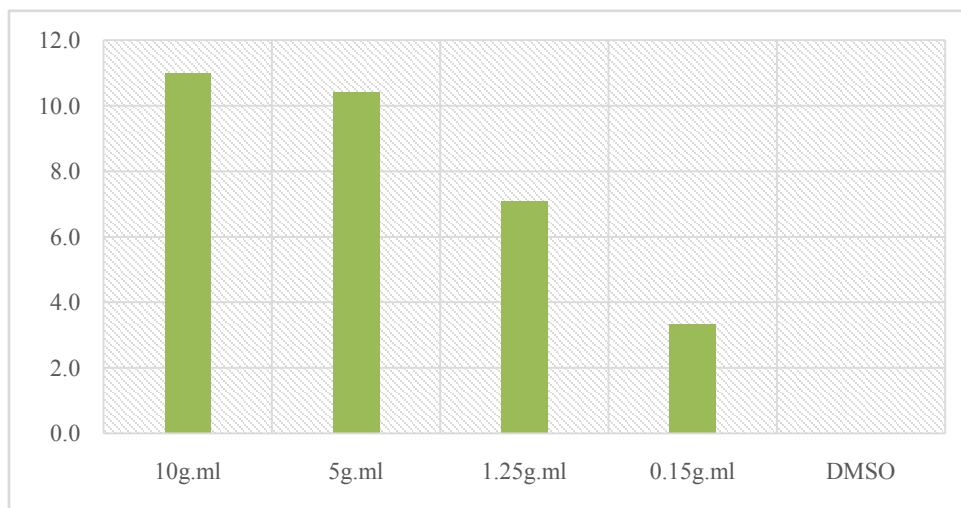


Figure 2 : Antibacterial effect of different concentration

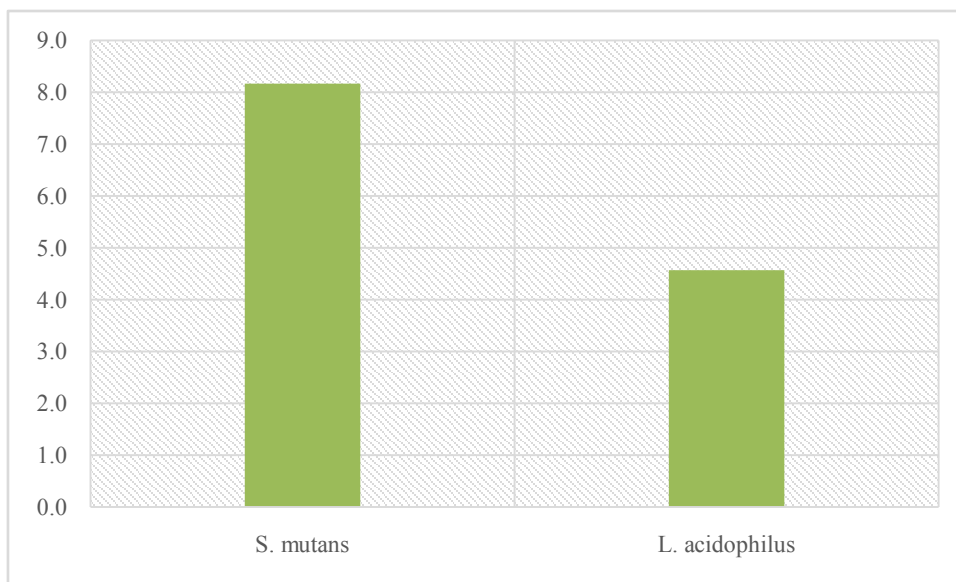


Figure 3 : Sensitivity of *S. mutans* and *L. acidophilus*

Result showed combination of peels and arils have greater antibacterial effect than pure peel extract (Figure 4). Also result showed combination of peels and arils have greater antibacterial effect on *L. acidophilus* in

comparison with pure peel extract. Also result showed pure peel extract has greater antibacterial effect on *S. mutans* in comparison with combination of peel: arils extract (Figure 5).

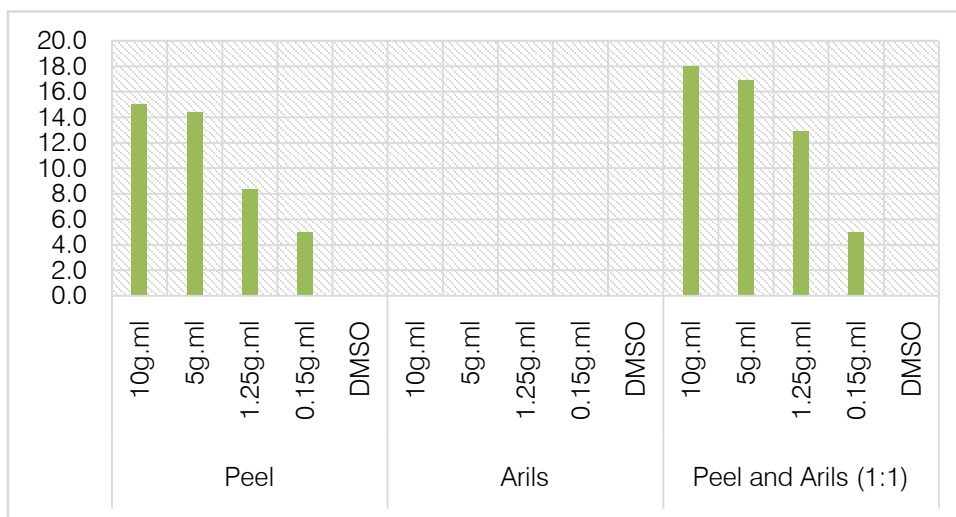


Figure 4 : Sensitivity of bacteria to peel and arils extract

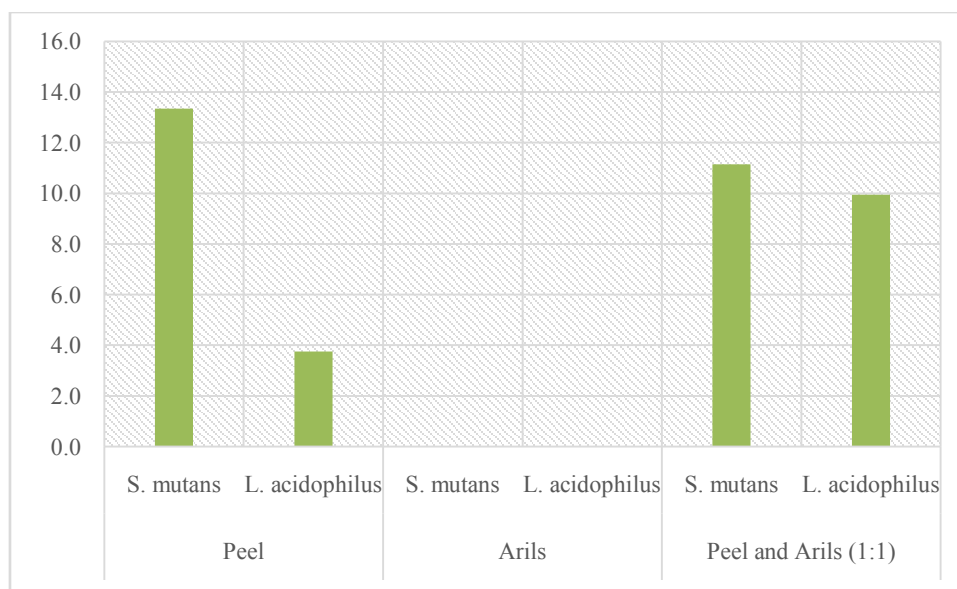


Figure 5 : Sensitivity of bacteria to peel and arils extract

#### IV. DISCUSSION

Result showed combination of peels and arils extract has greater inhibitory effect. Arils have no inhibitory effect against selected organisms. Result showed combination of peels and arils have greater antibacterial effect than pure peel extract. Also result showed combination of peels and arils have greater antibacterial effect on *L. acidophilus* in comparison with pure peel extract. Also result showed pure peel extract has greater antibacterial effect on *S. mutans* in comparison with combination of peel: arils extract.

Arils of pomegranate, contains 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin. Also arils contain organic acid such as ascorbic acid, citric acid, and malic acid. Arils contain bioactive compounds such as phenolics, flavonoids and principally anthocyanins. The seeds are a rich source of total lipids. (Aviram et al., 2000; Tezcan et al., 2009). The arils contain less chemical substances in comparison with pomegranate peel.

Pomegranate peel is rich in hydrolyzable tannins like punicalin, pedunculagin, and punicalagin (Seeram et al., 2005). Peel is rich in esters of hexahydroxydiphenic acid and glucose or quinic acid (Clifford et al., 2000). Also pomegranate peel contains hydroxybenzoic acids such as gallagic, glycosides (Amakura et al., 2000). Pomegranate peel contains anthocyanidins which are principally cyanidin, pelargonidin, and delphinidin (Noda et al., 2002). Pomegranate peel contains flavonoids such as kaempferol, luteolin, and quercetin (Van Elswijk et al., 2004).

#### V. CONCLUSION

Combination of (Peel: arils) extract has greater antibacterial effect than the pure extract of the arils and

the peels. Also result confirmed arils were not effective in the inhibition of *S. mutans* and *L. acidophilus*.

#### VI. RECOMMENDATIONS

Further study on antibacterial effect of seed extract in combination with juice and peels is recommended. Also further study on antibacterial effect against wider range of oral bacteria is recommended.

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