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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 grater antibacterial effect on S. mutans in comparison with combination of peel and arils extract.

Index terms— pomegranate (punica granatum) peels, arils, S. mutans, L. acidophilus.

1 Evaluation of the Antibacterial Activity in Pomegranate Peels and Arils by S. Mutans and L. Acidophilus

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 grater antibacterial effect on S. mutans in comparison with combination of peel and arils extract.

2 Introduction

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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-3glucose, quercetin, gallic acid, pelargonidin-3galactose and myricetin which has antibacterial activity. Although studies show *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.

3 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.

4 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 shaken 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.

5 d) Measurement of Antibacterial Activity 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.

6 III.

7 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*. 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. 30% 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. Extract has greater inhibitory effect. Result showed Arils has no inhibitory effect against selected organisms. But result showed peels have inhibitory effect.

8 Figure 1 : Antibacterial effect of different extracts

Result showed by decrease in concentration of peel extract antibacterial effect of peel was decreased (Figure ??) and *S. mutans* is more sensitive to peel extract than *L. acidophilus* (Figure ??). 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 ??).

9 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; Ezzanet 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.

10 Conclusion

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

11 VI.

12 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. ¹



Figure 1: Fatemeh

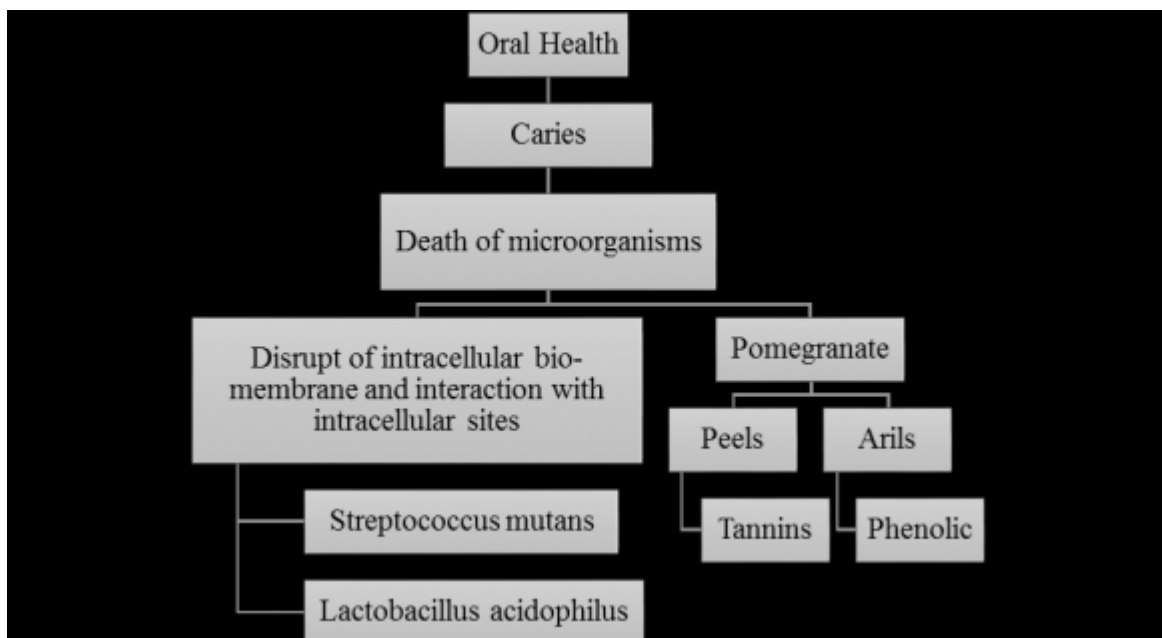


Figure 2:

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