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4

5 **Abstract**

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

22

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

24 **1 Evaluation of the Antibacterial Activity in Pomegranate Peels 25 and Arils by S. Mutans and L. Acidophilus**

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

39 **2 Introduction**

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42 Research showed low concentration of P. granatum extract led to delay in S.aureus growth, while in a higher
43 concentration of P.granatum extract, growth of S.aureus was eliminated (Braga et al., 2005). P. granatum also

9 DISCUSSION

44 has antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus* and *Klebsiella* (Fawole et al., 2012). Investigation
45 on the chemical composition of pomegranate fruit led to identification of cyanidin-3glucose, quercetin, gallic acid,
46 pelargonidin-3galactose and myricetin which has antibacterial activity. Although studies shows *Punica granatum*
47 has antibacterial potential against few bacterial strains but there is lack of investigation on antibacterial property
48 of *Punica granatum* against oral bacterial. Also the indiscriminate use of antibiotics led to an increase in antibiotic
49 resistance between different microorganisms. This situation shows the need for development of novel antibiotics
50 ??Das et al., 2010).

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

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

57 3 b) Preparation of Bacterial strain

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

60 4 c) Methods of Extraction

61 Fresh pomegranate arils and peels were cleaned and separated. The peels and arils separately grounded blender.
62 Fifty grams of blended peels or arils placed in 250ml Erlenmeyer flasks, followed by adding 100 ml of 95% ethanol.
63 The flasks shacked at room temperature for 18 h prior to filtration with Whatman paper. The filtrated mixtures
64 were concentrated under reduced pressure using rotary evaporator at 40 °C. These crude extracts were kept at 4
65 °C until use.

66 5 d) Measurement of Antibacterial Activity e) Statistical Analysis

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

70 6 III.

71 7 Results and Analysis

72 Result of ANOVA analysis showed there is significant difference between different concentration of different
73 extract (P<0.01). Also result showed there is significant difference on inhibition of *S. Various* concentrations of
74 the peel, arils and peel and arils mixture (1:1) prepared by dissolving in Dimethyl Sulphoxide (DMSO) to obtain
75 a final concentration of 10g.ml, 5g.ml, 2.5g.ml and 1.25g.ml against the test organisms. The test inoculums
76 swabbed uniformly onto the MRS agar plates and wells of diameter 8mm were punched out in each plate. 30?1
77 of each of these extracts were pipetted out into these wells, the plates incubated upright at 37°C overnight.
78 Dimethyl sulfate used as negative control. The sensitivity of bacterial strains to aqueous and alcoholic extracts
79 of the different extract of *Punica granatum* calculated by measuring the diameter of inhibition zone. Bacteria
80 showing a clear zone of inhibition >4mm considered to be sensitive. Experiments performed in triplicates for
81 each combination of extract and the bacterial strain. extract has greater inhibitory effect. Result showed Arils
82 has no inhibitory effect against selected organisms. But result showed peels have inhibitory effect.

83 8 Figure 1 : Antibacterial effect of different extracts

84 Result showed by decrease in concentration of peel extract antibacterial effect of peel was decreased (Figure ??)
85 and *S. mutans* is more sensitive to peel extract than *L. acidophilus* (Figure ??). Result showed combination of
86 peels and arils have greater antibacterial effect than pure peel extract (Figure 4). Also result showed combination
87 of peels and arils have greater antibacterial effect on *L. acidophilus* in comparison with pure peel extract. Also
88 result showed pure peel extract has greater antibacterial effect on *S. mutans* in comparison with combination of
89 peel: arils extract (Figure ??).

90 9 Discussion

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

96 Arils of pomegranate, contains 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin.
97 Also arils contain organic acid such as ascorbic acid, citric acid, and malic acid. Arils contain bioactive compounds

98 such as phenolics, flavonoids and principally anthocyanins. The seeds are a rich source of total lipids. ??Aviram
99 et al., 2000; ??ezcanet al., 2009). The arils contain less chemical substances in comparison with pomegranate
100 peel.

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

106 V.

107 10 Conclusion

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

109 11 VI.

110 12 Recommendations

111 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

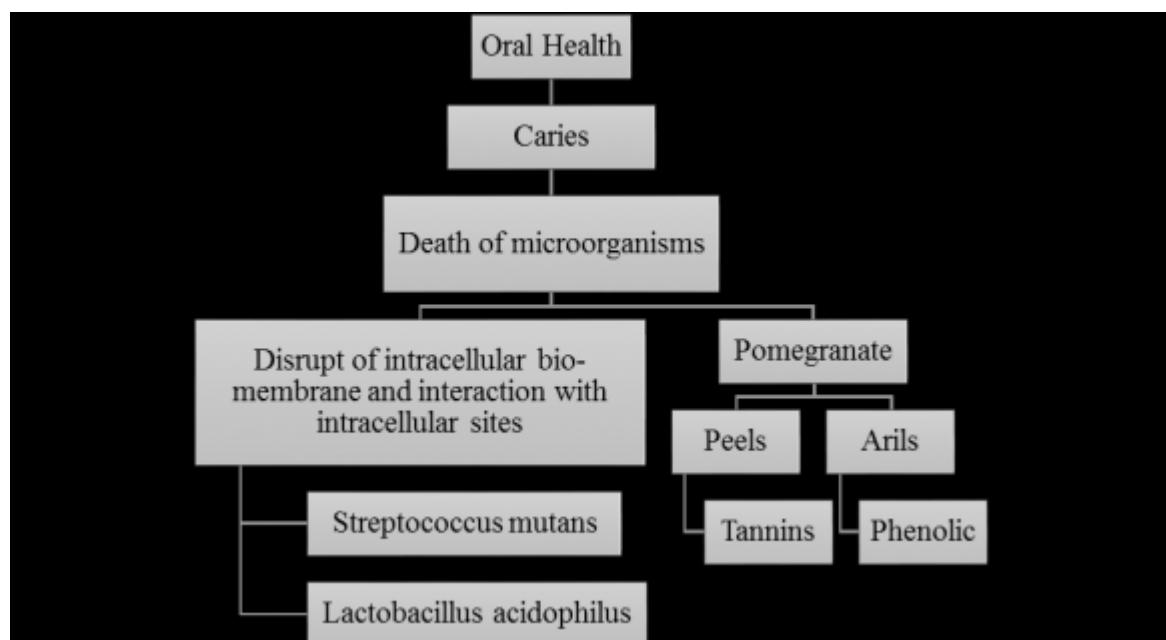


Figure 2:

112

12 RECOMMENDATIONS

113 [Peel Arils Peel and Arils (1:1) Nutr] , *Peel Arils Peel and Arils (1:1) Nutr* 136 p. .

114 [Das et al.] , K Das , R K S Tiwari , D K Shrivastava . *Journal of Medicinal Plants Research* 4 (2) p. 18.

115 [Naz et al. (2007)] ‘Antibacterial activity directed isolation of compounds from Punicagranatum’ S Naz , R
116 Siddiqi , S Ahmad , S A Rasool , S A Sayeed . *J Food Sci* 2007 Nov. 72 (9) p. .

117 [Fawole et al. (2012)] ‘Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel
118 methanolic extract’ O A Fawole , N P Makunga , U L Opara . 10.1186/1472-6882-12-200. *BMC Complement
119 Altern Med* 2012 Oct 30. 12 p. 200.

120 [Noda et al. ()] ‘Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin,
121 cyanidin, and pelargonidin’ Y Noda , T Kaneyuka , A Mori , L Packer . *J Agric Food Chem* 2002. 50
122 p. .

123 [Tezcan et al. ()] ‘Antioxidant activity and total phenolic, organic acid and sugar content in commercial
124 pomegranate juices’ F Tezcan , M Gültekin-Özgüven , T Diken , B Özçelik , F B Erim . *Food Chem* 2009.
125 115 (3) p. .

126 [Olapour and Najafzadeh ()] ‘Evaluation analgesic, anti-inflammatory and antiepileptic effect of hydro alcoholic
127 peel extract of Punica granatum (Pomegranate)’ S Olapour , H Najafzadeh . *Asian Journal of medical
128 Sciences* 2010. 2 (6) p. .

129 [Amakura et al. ()] ‘High-performance liquid chromatographic determination with photodiode array detection
130 of ellagic acid in fresh and processed fruits’ Y Amakura , M Okada , S Tsuji , Y Tonogai . *J Chromatog A*
131 2000. 896 p. .

132 [JayP (1938)] ‘Lactobacillus Acidophilus and Dental Caries’ JayP . *Am J Public Health Nations Health* 1938
133 June.

134 [Braga et al. ()] ‘Pomegranate extract inhibits *Staphylococcus aureus* growth and subsequent enterotoxin pro-
135 duction’ L C Braga , J W Shupp , C Cummings , M Jett , J A Takahashi , L S Carmo , E Chartone-Souza ,
136 A M Nascimento . *J Ethnopharmacol* 2005.

137 [Aviram and Dornfeld ()] ‘Pomegranate juice consumption inhibits serum angiotensin-converting enzyme activity
138 and reduces systolic blood pressure’ M Aviram , L Dornfeld . *Atherosclerosis* 2001. 158 (1) p. .

139 [Seeram et al. ()] *Pomegranate juice ellagitannin*, N P Seeram , S M Henning , Y Zhang , M Suchard , Z Li , D
140 Heber . 2006.

141 [Van Elswijk et al. ()] ‘Rapid dereplication of estrogenic compounds in pomegranate (Punicagranatum) using
142 on-line biochemical detection coupled to mass spectrometry’ D A Van Elswijk , U P Schobel , E P Lansky ,
143 H Irth , J Van Der Greef . *Phytochem* 2004. 65 (2) p. .

144 [Lehl et al. ()] ‘Relationship between cariogenic diet and dental caries as evaluated from a 5-day diet diary in
145 4-12 yr old children’ G Lehl , K Bansal , R Sekhon . *J Indian SocPedodPrev Dent* 1999. 17 p. .

146 [Clifford and Scalbert ()] ‘Review: ellagitannins-nature, occurrence and dietary burden’ M N Clifford , A
147 Scalbert . *J Sci Food Agric* 2000. 80 p. . (Combination of (Peel: arils)