

Antibacterial Activity of *Camellia Sinensis* Leaf Extract Against Wound Pathogenic Bacteria

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Introduction

Tea is one of the most widely consumed beverages in the world today, second only to water. Its medicinal properties have also been widely explored and the most significant effects on human health have been observed with the consumption of green tea (Cabrera *et al.*, 2006). Green tea and fresh tea leaf extract have same chemical compositions as well as the same therapeutic benefits. Thus, the present study was carried out to investigate the anti-microbial activity of methanolic extracted from fresh tea leaves against wound pathogenic bacteria with compare to the commercially available antibiotic called "Providone iodine (PVPI)" which is commonly used against wound pathogens, to see the potential therapeutic effect of tea leaf extract to formulate a natural antibiotic treatment for wound healing through a separate investigation as a value addition strategy in tea.

Methodology

Randomly collected fresh tea leaves were oven dried and grinded into powdery form using mortar and pestle. Then 12.5 g of grinded tea leaves were soxhlet extracted using 125 ml of 95 % methanol and the antibacterial activity of tea leaf extract was assessed against five most common wound bacteria; *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Eschirechia coli*, *Pseudomonas aureginosa* and *Enterococcus faecalis* via antibacterial susceptibility test and minimum inhibitory concentration (MIC) using paper disc diffusion method described by Mbata etal (2008) and broth dilution method described by Baron and Finegold (1990) respectively. PVPI and absolute methanol were used as positive and negative controls for antibacterial susceptibility test and three plates were duplicated in all experiments. MICs for tea leaf extract and PVPI were comparatively analyzed using nutrient both as its control. All the data were statistically analyzed.

Results and Discussion

The tea leaf extract has been found to exhibit an overall superiority in its antimicrobial activity compared to the PVPI which is recognized as a major active antibiotic agent in commonly used for wound treatments against the test organisms used in the experiment.

Table 1: Results for the antibacterial susceptibility test

Test Organism	Mean ZOI (mm) \pm SD		
	Tea Leaf Extract	Absolute Methanol	Providone Iodine
<i>S. aureas</i>	20.3 \pm 0.6	0	13.3 \pm 0.6
<i>S. pyogenes</i>	20.6 \pm 1.2	0	13.3 \pm 0.6
<i>E. coli</i>	19.6 \pm 0.6	0	08.3 \pm 0.6
<i>P. aureginosa</i>	22.0 \pm 3.5	0	08.3 \pm 0.6
<i>E. faecalis</i>	14.6 \pm 0.6	0	10.6 \pm 0.6

Table 1 shows the zones of inhibition (ZOI) after 24 hours for each test organisms. The data represent the mean \pm SD for three measurements. The mean values of tea leaf extract for each bacterial strain were showed a significantly higher ($P < 0.05$) activity compared to PVPI as analyzed by Two-Sample T-Test.

Table 2 shows the potency of tea leaf extract and PVPI to be either antibacterial resistance or susceptible according to the zone diameter interpretation standards approved by Clinical Laboratory Standards Institute (CLSI).

Table 2: Zone diameter interpretation for the test results

Test Organism	Zone Diameter Interpretation		
	Tea Leaf Extract	Absolute Methanol	Providone Iodine
<i>S. aureas</i>	S	R	I
<i>S. pyogenes</i>	S	R	I
<i>E. coli</i>	S	R	R
<i>P.aureginosa</i>	S	R	R
<i>E. faecalis</i>	I	R	I

S - Antibacterial Susceptible (16 mm or more), I – Intermediate (11 – 15 mm), R - Antibacterial Resistance (10 mm or less)

The result in the Table 2 shows that all the test organisms were sensitive to the tea leaf extract. Only *Enterococcus faecalis* has intermediate resistance to the tea leaf extract. Similarly, *S. aureus*, *S. pyogenes* and *E. faecalis* were showed intermediate resistance while *E. coli* and *Pseudomonas aureginosa* were not sensitive to the PVPI. It implies that the PVPI's inhibitory activity only compatible with Gram positive bacteria but not with Gram negative bacteria where as tea green leaf extracts agreeable to both Gram positives and negatives bacteria.

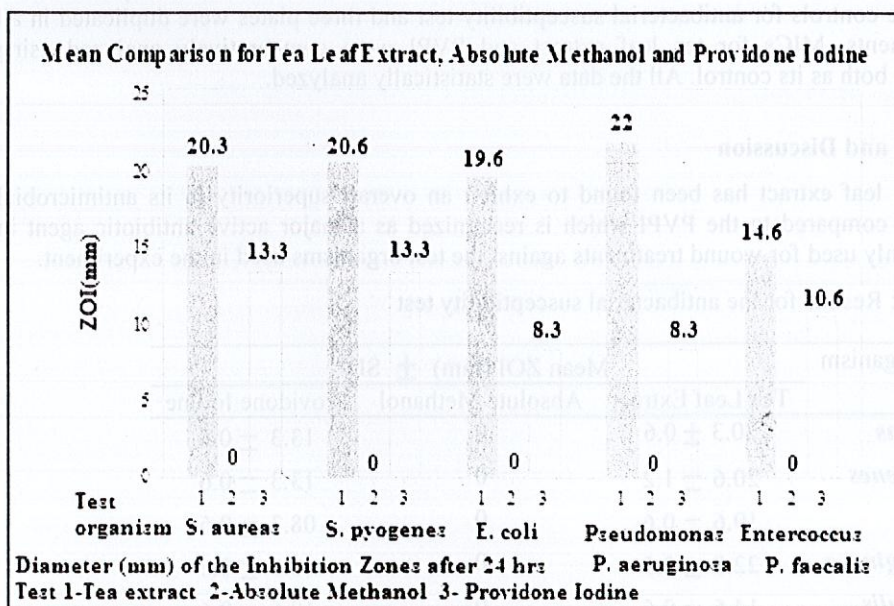


Figure 1: Zone of inhibition to various test organisms

Figure 1 shows the mean comparisons of tea leaf extract, solvent used and the positive control (absolute methanol and PVPI respectively) as analyzed by the Descriptive Statistical Analysis.

Table 3 shows the experiment ascertained tea leaf extract required less MIC value (0.008 mg/ ml) to kill Gram negative bacteria than the MIC required by the PVPI (≥ 0.02 mg/ ml). Both tea and PVPI bared same MIC value (0.004 mg/ ml) for Gram positive wound pathogenic bacteria. This implies tea required fewer compounds to show a greater antibacterial response.

Table 3: MIC value of tea leaf extract and PVPI for test organisms

Test Organism	MIC* ($\mu\text{g}/5\text{ ml}$)	
	Tea Leaf Extract	Providone iodine
<i>Staphylococcus aureas</i>	20	20
<i>Streptococcus pyogenes</i>	20	20
<i>Escherichia coli</i>	40	100
<i>Pseudomonas aeruginosa</i>	40	>100
<i>Enterococcus faecalis</i>	20	60

MIC is the concentration of a compound required for killing of bacteria. So, MIC* is the concentration of the first test tube which indicated clear suspension without any growth of the test organism after 24 hours incubation at 37 °C.

The highest antimicrobial activity of tea is due to presence of catechins and polyphones which can cause damages to bacterial cell membrane (Houghton *et al.*, 2005). Gram negative bacteria required higher concentrations because Gram negative bacteria have more complex cell wall than Gram positives (Aulton, 2002).

Conclusion

The results showed that the tea leaves extract were shown strong antibacterial activity and can be used as potential source for the formulation of a therapeutic agent to control wound pathogenic bacteria in wound infections.

References

- Aulton, M. E. 2002. The science of dosage form design, 2nd edition, Churchill Livingstone, Pp. 503-504. Edinburgh
- Baron, J.E. and Finegold, S.M. 1990. Method for testing antimicrobial effectiveness. In: Bailey Scotts Diagnostic Microbiology. Mosby, C.V. (ed.), Missouri. pp 171 – 194
- Cabrera, C., Artacho, R. and Giménez, R.2006. Beneficial effects of green tea: a review. J Am Coll Nutr 25:79-99
- Clinical Laboratory Standards Institute. 2006. Performance standards for antimicrobial disk susceptibility tests; Approved standard—9th ed. CLSI document M2-A9. 26:1. Clinical Laboratory Standards Institute, Wayne, PA
- Houghton, P. J., Hylands, P. J. and Mensah, A.Y. 2005. In vitro tests & ethnopharmacology investigations: wound healing as an example. J. Ethnopharmacol, 100: 100 – 107
- Mbata, T. I., Debiao, L. U. and Saikia, A. 2008. Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*, ISSN 1684- 5315