



Development of an Antimicrobial Topical Skin Cream with Using Wood Apple (*Limonia acidissima* L.) Pulp Against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*[#]

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ABSTRACT

Skin inhabiting microbes mostly non-pathogenic and commensals on the skin. Among them *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* often responsible for symptomatic skin infections. Cellulites, impetigo, ecthyma, red lesions and candidiasis are some of the common microbial skin diseases caused by skin pathogens. Wood apple (*Limonia acidissima* L.) is an underutilized fruit in Sri Lanka and, all parts of this plant have medicinal value. Considering the high therapeutic value, the aqueous extract of wood apple pulp was used. Antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* was evaluated by using the standard well diffusion method. Above tested bacterial and fungal species were suspended in 3 mL of sterile distilled water separately and 10⁸ CFU/mL population count was prepared. Aliquots of 100 µL of each suspension were inoculated and uniformly spread on Muller Hinton agar plates separately in triplicates. Wells were filled aseptically placing 50 µL of wood apple extracts, antimicrobial compounds specifically as the positive control for bacteria and fungi and sterile distilled water as the negative control. The plates were incubated at 37 °C for 24 - 48 hours and the diameter of the growth inhibition zones around the wells were measured. Furthermore, minimum inhibition concentrations of each extract were also evaluated. Comparisons were performed using one-way ANOVA followed by Tukey's Pairwise Comparisons. The antimicrobial topical skin cream was prepared using 0.5 g mL⁻¹ of wood apple pulp with sterile distilled water, mixed with the formulated standard ingredients. The results revealed that the aqueous extracts of wood apple pulp concentrations range from 0.15 g mL⁻¹ to 1g mL⁻¹ showed the antimicrobial potential of above skin pathogens variably ranged inhibition zone diameter 16 mm to 34 mm. The antimicrobial effect of the formulated cream against these pathogens was repeatedly tested. Further testing procedures needed to be followed before recommending wood apple topical antimicrobial skin cream as a marketable product.

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Introduction

The skin microbiome are the microorganisms that inhabited on the skin (Fuchs and Raghavan, 2002; Farahmand, 2020). Although the skin diseases are very common in Sri Lanka, people have not been considered them as a significant problem. Most of the skin inhabited microbes are found in the superficial layers of the epidermis and the upper parts of hair follicles (Chiller et al., 2001; Grice et al., 2009). They are usually non-pathogenic, and either commensals or mutualists (Higaki et al., 2008). However, resident microbes can cause skin diseases and controlling such microorganisms are important in preventing the skin diseases and their transfer to the others. *Propionibacterium* and *Staphylococcus* spp. are common in sebaceous areas of skin (Farahmand, 2020).

Staphylococcus aureus, a Gram-positive bacterium and commonly find in the lesions of various skin diseases, and its significance in the pathogenesis of many skin diseases is well established (Farahmand, 2020). Many reports have referred to the prevalence of methicillin-resistant *S. aureus* (MRSA), which is a frequent cause of dermatological problems (Grice et al., 2009). *Pseudomonas aeruginosa* is an aerobic Gram-negative bacterium which is an opportunistic pathogen (Aendeker et al. 2005). *Candida albicans*, are commensal yeast of the mouth, gastrointestinal tract, skin, and vagina of healthy individuals (Conti and Gaffen, 2015).

Wood apple (*Limonia acidissima* Groff) is a slow-growing, medium sized tree, widely found in India, China, Nepal, Sri Lanka, Myanmar, Pakistan, and Bangladesh (Kumar and Rao, 2011). The fruit is round 5-12.5 cm wide with a woody, hard rind which is difficult to crack. The pulp of *L. acidissima* is sticky, brown, and aromatic. It is odorous, resinous, astringent, acid or sweetish, with numerous small, white seeds scattered through it (Bagul et al. 2019). The different parts of the plant include its roots, fruits, bark and the leaves which are used for various therapeutic purposes. It was reported that wood apple possesses a very strong antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis* and *Proteus mirabilis* (Senthilkumar and Venkatesalu, 2013). It was found that both pulp and rind inhibit the activity of various microbes and thus, prevents the onset of diseases caused by bacteria and fungi (Bagul et al. 2019). This property of wood apple pulp may be due to the abundance of polyphenols and flavonoids (Bagul et al. 2019).

Materials and Methods

Sample collection and extraction of wood apple pulp

Wood apple samples were collected from three different locations at Mihintale, Sri Lanka and stored in a refrigerator at 4°C. The ripe wood apples were washed with distilled water to remove all the impurities around the fruit. After that by using a pestle, the rind of the fruits was broken, seeds were removed from the pulp and 1 g mL⁻¹, 0.5 g mL⁻¹, 0.4 g mL⁻¹, 0.33 g mL⁻¹, 0.25 g mL⁻¹ and 0.15 g mL⁻¹ concentrations of wood apple pulp extracts were prepared using sterile distilled water.

Subculture of skin pathogens

Pure cultures of skin bacterial pathogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and fungal pathogen *Candida albicans* were obtained from the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka. The bacterial strains of *S. aureus* and *P. aeruginosa* and a yeast *C. albicans* were sub-cultured in mannitol salt agar and King's B agar and Sabouraud dextrose agar respectively. Subcultures were incubated at 37°C temperature and the stock cultures were maintained at 4°C and sub-cultured at regular intervals (Pfaller et al. 1997).

Determination of antimicrobial activity against tested skin pathogens

The antimicrobial activity was determined by using the standard agar well diffusion method (Ericsson and Sherris 1971; Valgas et al., 2007). Each tested bacterial strain suspended in 3 mL of sterile distilled water with turbidity optically comparable to that of the 0.5 McFarland standard (1.5×10⁸ CFU/mL) and 100 µL aliquots of each suspension was inoculated and uniformly spread on the surface of Muller Hinton agar plates in triplicates separately. Thereafter, 6 mm wells were made at equidistance. An amount of 50 microliter of wood apple pulp extract at different concentrations was dispensed into each well. The plates were allowed to diffuse for 30 minutes and incubated at 37 °C for 24 hours and zone of inhibition was measured. Each test was performed in duplicates with suitable

controls. The similar procedure was followed for *C. albicans* with using Sabouraud dextrose agar and diameter of the inhibition zones (in mm) were measured after 48 hours (Magaldi et al. 2004). Chloramphenicol and Gentamycin were used as a positive control for tested bacteria and Fluconazole for *Candida* respectively.

Statistical analysis

Wood apples were selected from three localities and antimicrobial testing was also done in triplicates. The diameter of the inhibition zone was expressed as means ± standard deviation (SD). Statistical analysis was designed as two-factor factorial Completely Randomized Design (CRD) using the SAS program (Version 9.0, SAS Institute Inc. USA). Means were compared using Tukey's simultaneous test set at p < 0.05.

Preparation of antimicrobial topical skin cream

Antimicrobial topical skin cream was formulated (Chen et al. 2016) with the appropriate concentration of extract which was determined by the testing of antimicrobial activity. Antimicrobial topical skin cream was formulated and developed with wood apple pulp (Table 1) for controlling *S. aureus*, *P. aeruginosa* and *C. albicans*. Antimicrobial activity of the skin cream was also tested for above skin pathogens by repeating standard well diffusion assay.

Table 1. Formulation of antimicrobial topical skin cream

Compound	Function	(%)
Ultras 21	Thickening agent	0.5
Glycerin	Moisturizing agent	5
EDTA	Chelating agent	0.1
Propylene glycol	Stabilizer, Co-solvent	2
Wood apple extract	Active ingredient	25
Sterile distilled water		63.4
Novemer	Thickening agent	1.5
NaOH (18%)	PH adjustment	0.5
Neolan PE	Preservative	1
Fragrance		1

Results and Discussion

Antimicrobial activity of wood apple pulp extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

It was revealed that 0.5 mg mL⁻¹ concentration of wood apple pulp extract showed the statistically significant (p < 0.05) inhibition on the growth of *S. aureus* (Figure 01). However, the reduced diameter of the inhibition zone for *S. aureus* in the present study may be due to the aqueous extract. Furthermore, statistical analysis revealed that there was no significant difference (P < 0.05) observed in the growth inhibition of *S. aureus* with 0.5 mg mL⁻¹ wood apple pulp and the antibiotic chloramphenicol (Figure 1). According to Ponnuraj et al. (2015) the methanol extract of the fruit pulp was also shown the antibacterial activity against *S. aureus* (21.6 ± 0.23 mm).

Although most of the tested wood apple concentrations were inhibited the growth of *P. aeruginosa*, 0.5 mg mL⁻¹ showed the highest level of inhibition (Figure 2). It was

reported by Sonawane and Arya, (2013), that the *L. acidissima* pulp contained phenolic content (38.61 mg/g), 3.41 mg/g ascorbic acid, and possess antibacterial characteristics against *Pseudomonas aeruginosa*. Furthermore, the protein hydrolyzate of *L. acidissima* seeds showed antimicrobial activity at 300 (mg/mL) against *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* (Jamil et al. 2019).

The wood apple pulp concentration of 0.5 mg mL⁻¹ also showed the highest level of growth inhibition of *C. albicans* (Figure 3). The topical antimicrobial skin cream was formulated with using 0.5 mg mL⁻¹ wood apple pulp. Antimicrobial activity of the skin cream was shown similar growth inhibition of tested skin pathogens (Widyawati et al., 2014). Therefore, the results revealed that tested topical

skin cream was potentially effective in suppressing tested skin pathogens. However, before recommending the antimicrobial skin cream, series of other testing procedures should be followed (Martorell et al. 2020).

The GC/MS screening results were reported by Valgas et al. (2007) denoted that the presence of hydrocarbons, sterols, aldehydes, carboxylic acids and their esters, phenolic acids, and flavonoids may contribute towards the antimicrobial properties of *L. acidissima*. Furthermore, Vijayvargia et al. (2014), revealed that the extracts n-hexane, ethyl acetate and ethanol has not changed the effectivity of antimicrobial activity of *L. acidissima* fruit peel. The secondary metabolites of the most plants possess various anticancer, anti-inflammatory, and antioxidant properties (Jiang et al. 2019).

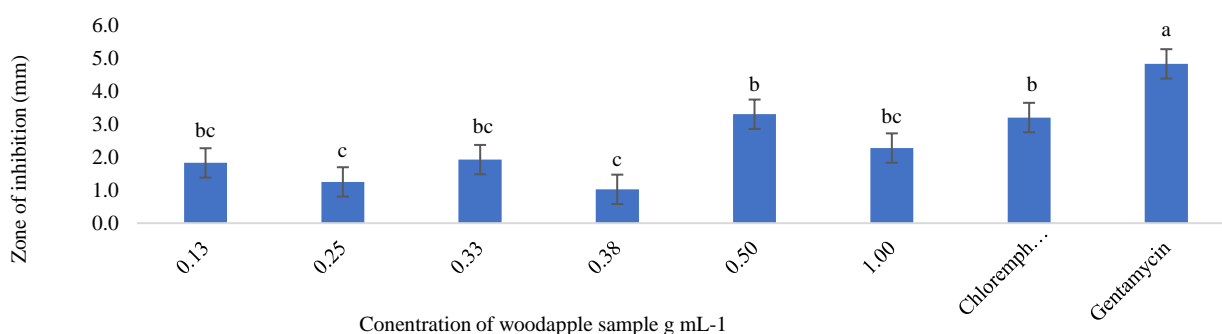


Figure 1. The diameter of the growth inhibition zone (mm) around the wells of wood apple pulp extract concentrations in g mL⁻¹ on lawn of *Staphylococcus aureus*. Error bars indicate 95% confidence intervals. Means that do not share a same letter differ at P=0.05.

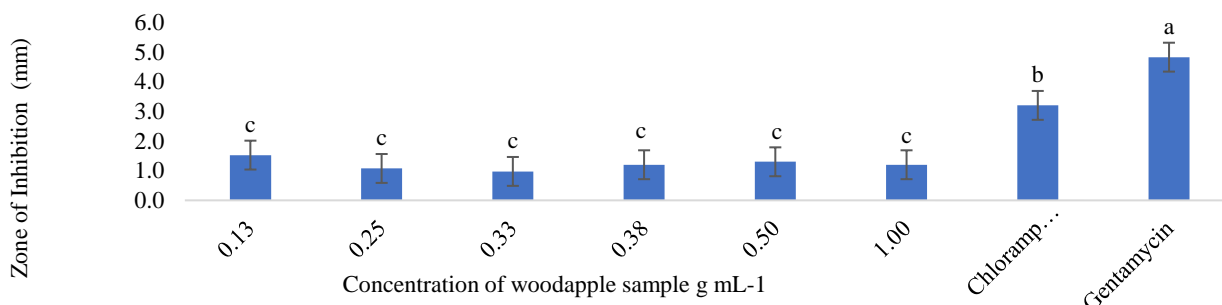


Figure 2. The diameter of the growth inhibition zone (mm) around the wells of wood apple pulp extract concentrations in g mL⁻¹ on lawn of *Pseudomonas aeruginosa*. Error bars indicate 95% confidence intervals. Means that do not share a same letter differ at P=0.05.

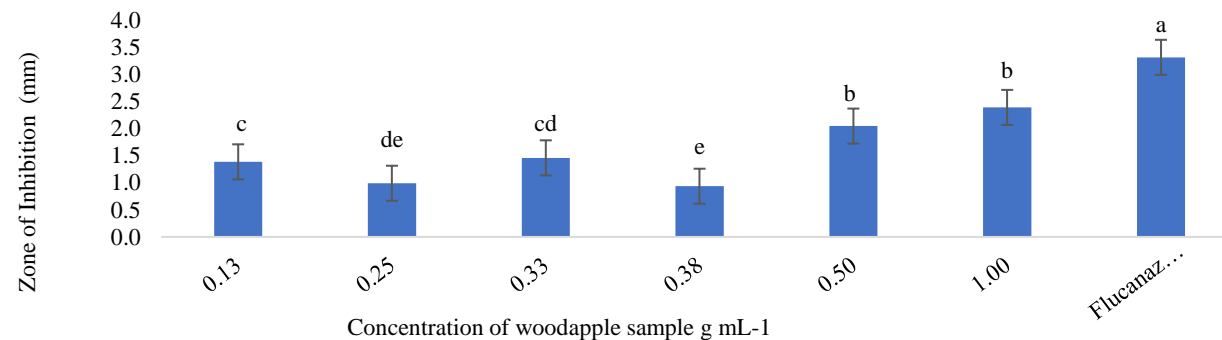


Figure 3. The diameter of the growth inhibition zone (mm) around the wells of wood apple pulp extract concentrations in g mL⁻¹ on yeast, *Candida albicans*. Error bars indicate 95% confidence intervals. Means that do not share a same letter differ at p = 0.05.

Conclusion

Ripe wood apple pulp of 0.5 g mL⁻¹ in sterile distilled water is potentially effective in controlling skin microbial pathogens such as, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Despite the synthetic antimicrobial treatments, natural topic skin cream can be used. Thus, there is enormous scope for future research and further pharmacological investigations on *Limonia acidissima* pulp as the antimicrobial topic skin cream, following appropriate testing processes, before warranted for industrial production.

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