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Antibacterial effects of *Curcuma* Species and *Vigna radiata* against MRSA, MRSE, and *Propionibacterium acnes.*

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Abstract

Bacterial infections can be treated by antibiotics, but bacteria are developing resistance against antibiotics which has led to a global threat. Antibiotic-resistant bacteria will result in hard-to-treat infections. These will increase medical costs, lengthen hospital stays and increase in mortality rate. Many studies are searching for a promising solution for antibiotic resistant issues. In this research, three herbal plants, *Curcuma aromatica, Curcuma zedoaria,* and *Vigna radiata* were used to study the antibacterial activity against ten gram positive strains of bacteria, which were *Staphylococcus aureus, Staphylococcus aureus* FB408/B, *Staphylococcus epidermidis*, *Staphylococcus epidermidis* S15B/A and *Propionibacterium acnes* as well as three strains of Methicillin Resistant *Staphylococcus aureus* (MRSA), which were MRSA positive control, MRSA S16B/A, MRSA F13, and as well as two strains of Methicillin Resistant *Staphylococcus epidermidis* (MRSE), which were MRSE S19 and MRSE S23. These three plants were selected for this study because they are commonly using in Chinese and Indian traditional medicines as wound healing agents and bath scrubs. Phytochemicals from *C. aromatica, C. zedoaria*, and *V. radiata* were extracted with 85% ethanol using a Soxhlet extractor and concentrated using a rotary evaporator. Phytochemical screening was conducted and analyzed the presence of plant secondary metabolites that are involved in antibacterial activity. All the tested plant extracts contained terpenoids, phenols and tannins in general. These ethanolic extracts were tested against the ten gram-positive isolates. All plant extracts inhibited bacterial growth with variable potency. Synergistic effects were observed in combination of the three plants extracts when tested with *S. aureus* FB408/B and MRSA F13. Furthermore, when tested *C. aromatica* with *C. zedoaria,* showed a higher antimicrobial effect against the MRSA positive control in combination, than individually. The combination of *C. zedoria* with *V. radiata* on the other hand showed higher activity in *P. acnes.* Even though synergistic effects were observed, individual plant extract of *C. aromatica* and *C. zedoaria* have shown the greatest antibacterial activity against most of the tested isolates. The results in this study can be used to propose an effective facial product with *C. aromatica* extracts to inhibit activity of common skin pathogens and antibiotic resistant bacteria such as MRSA and MRSE that are found on human skin.

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Keywords

Curcuma species, *Vigna radiata*, ethanolic extracts, antibacterial activity, phytochemicals

Introduction

One of the greatest public health pressures in the world is the evolving antimicrobial resistance in gram-positive bacterial infections (GPIs) which has led to an increase in mortality and morbidity rates (Kulkarni et al., 2019). *S. aureus* which is commonly found on skin, causes soft tissue and skin infections (Gompf, 2018) and has evolved into resistant strains which are not or less sensitive to many types of antibiotics such as methicillin, oxacillin, penicillin, and amoxicillin (Achek et al., 2018). *S. epidermidis* is commonly found in mucosal microflora and on healthy human skin. (Eslami et al., 2017). More recently, S. epidermidis escalated as a nosocomial pathogen which is also multidrug resistant *i.e.* Methicillin Resistant *Staphylococcus epidermidis* (MRSE). *P. acnes* which is also known as *Cutibacterium acnes*, commonly found in sebaceous sites, is essential in the maintenance of skin homeostasis and avoid any harmful pathogens from colonizing. However, *P. acnes* also behaves as an opportunistic pathogen in acne vulgaris. (Dreno et al., 2018). Hence, GPIs have become a challenging task to treat and manage.

Discovery of antibiotics have reduced mortality and morbidity rate, but indiscriminate use of antibiotics has elevated the incidence of resistant bacteria. (Kulkarni et al., 2019). This crisis has resulted in scientists turning to medicinal plant research (Gupta & Birdi, 2017). Medicinal plants contain secondary metabolites such as flavonoids, alkaloids, tannins, phenols, saponins, terpenoids and many more. (Gupta & Birdi, 2017). These metabolites show emerging paradigm of antimicrobial activity and resistance modifying capabilities by binding to proteins and inhibiting or modifying cellular interaction (Gupta & Birdi, 2017). Plant extracts which contain trans-cinnamaldehyde and terpenes also been proven to control biofilm formation in many pathogenic bacteria (Gupta & Birdi, 2017).

In the meantime, the rise of antibiotic-resistant bacteria, which has been observed over decades, and attempts to formulate newer antimicrobial agents with more efficacy level and less toxicity would be a rational solution. However, this is not a practical approach as it takes more than ten years to develop a new drug with little success. Hence, utilizing natural compounds such as plant extracts with antimicrobial activity would be a logical solution to help curb antibiotic resistance.

Thus, the objective of this study was to investigate the combinatorial and individual antibacterial effects of *C. aromatica*, *C. zedoaria* and *V. radiata* extracts against *S. aureus*, MRSA, *S. epidermidis*, MRSE and *P. acnes*, and suggest an effective plant extract(s) for use as a facial product to inhibit the growth of these gram positive bacteria which are common skin pathogens.

Methodology

Preparation of plant material

Dried tubers of *C. aromatic* and *C. zedoaria,* and the seeds of *V. radiata* were purchased from LotusMart in Petaling Jaya, Selangor. The plant materials were rinsed with deionised water and dipped in 85% (v/v) ethanol to remove dirt before being left to sun dry for 3 days. The plant materials were then mechanically ground into fine powder and stored at 4ºC until further use.

Ethanolic extraction using a Soxhlet apparatus

The powdered plant material, 30 grams, was filled into a thimble and placed into the Soxhlet extractor (Favorit brand) and extracted with 85% (v/v) ethanol for 4 hours at a temperature of approximately 69ºC. The extracts were then concentrated using a rotary evaporator (Ika Brand). The final, concentrated liquid product was adjusted to a standard concentration of 2.4 g/ml using 85% (v/v) ethanol before being dispensed into a sterile universal bottles and stored at 4ºC until further use (Redfern et al., 2014).

Phytochemical screening

Ethanol extracts of *C. aromatica*, *C. zedoaria,* and *Vigna radiata A. indica* were subjected to phytochemical screening including tests for alkaloids, phytosterols, fixed fats and oils, flavonoids, saponins and terpenoids as per the methodology cited in Thilagavathy et al., 2015.

Agar well diffusion assay

A suspension of each bacteria tested diluted obtain 5 x10^5 CFU/mL and spread onto Mueller-Hinton agar plates (OXOID) (Citron et al., 2005). Plant ethanolic extracts were dispensed into wells made in the agar plates. 85% (v/v) ethanol was used as a negative control. The diameter (mm) of the inhibition zone was measured after incubation at 37℃, overnight. The agar well diffusion assay was done in triplicates.

Statistical analysis

The mean value and standard deviation were calculated by using Microsoft Excel 2010. The IBM Statistical Package for Social Sciences (SPSS Version 20) were used to conduct. Two-way ANOVA test with a significance level of $P \leq 0.05$ was used on the data collected.

Results and Discussion

The qualitative phytochemical screening results for *C. aromatic*, *C. zrdoaria* and *V. radiate* are shown in Table 1. From the phytochemical screening conducted, saponins were not detected in the ethanolic extract of *C. aromatica*. Furthermore, alkaloids were not detected in *V. radiata*, while flavonoids were not detected in both *C. zedoaria* and *V. radiata*.

Table. 1 Phytochemical analysis (qualitative) of ethanolic extracts from *C. aromatic, C. zedoaria* and *V. radiata.*

Plant extracts	**Phytochemicals**					
	Tannins	Phenols	Alkaloids	Flavonoids	Saponin	Terpenoids
C. aromatica	✓	✓	✓	✓	X	✓
C. zedoaria	✓	✓	✓	X	✓	✓
V. radiata	✓	✓	X	X	✓	✓

Previous studies have shown that *C. aromatica* contains saponins when extracted with water but not detected when extracted with methanol or other polar solvents (Patil et al, 2015). Although saponins have polar properties and have been shown to be extracted by polar solvents such as ethanol, Widyawati et al. (2014) showed that that ethanolic extract yielded less concentration of saponins compared to water. From this study, we can deduce that the 85% ethanolic extract contained much lower concentrations of saponins which could be the reason why the qualitative assay used was unable to detect saponins from *C. aromatic* extracts.

The ethanolic extracts were then used singly and in combination to determine the antibacterial effects against the skin pathogens tested. The results revealed that individual and combined ethanolic plant extracts, inhibited tested isolates with variable potency. In general, the ethanolic extracts from *C. aromatica* were the most effective in inhibiting the growth of most of the bacterial isolates tested, compared to *C. zedoaria* and *V. radiata*. This could be correlated to the highest number of phytochemicals found in the *C. aromatica* extracts compared to the other two plant extracts. In the individual assays, *C. aromatica* effectively inhibited all the strains except for *S. epidermidis* and MRSE S15 B/A whereas *C. zedoaria* found to be effective against these two isolates against the other eight bacteria tested (Figure 1). Figure 1 showed that *C. zedoaria* was found to be more effective against *S. epidermidis* (20.7mm) and *S. epidermidis* S15B/A (31.33 mm) isolates. Individual extract of *C. aromatica* exhibited the maximum zone of inhibition (21.3 mm) in *P. acnes* isolate, which was greater than the positive control, cefazolin (10 mm). *V. radiata*, on the other hand, was fairly ineffective against all the isolates tested which could correlate with the fewer phytochemicals that were detected in this extract.

In addition, *C. aromatica* extracts inhibited the growth of MRSE S19 and S23 isolates, which were resistant to the antibiotic ceftriaxone, used as a control in this study. This result is promising in that the plant extracts were more effective compared to the antibiotic currently used in the treatment of infections caused by MRSE.

In general, individual plant extracts were found effective against these six isolates: *S. aureus*, MRSA F13, *S. epidermidis*, *S. epidermidis* S15 B/A, MRSE S23, MRSE S19 and *P. acnes*. An inference can be made that, even though the combination of plant extracts showed synergistic effects against some bacteria, but most of the bacterial growth were inhibited by individual plant extracts, specifically by *C. aromatica* extracts.

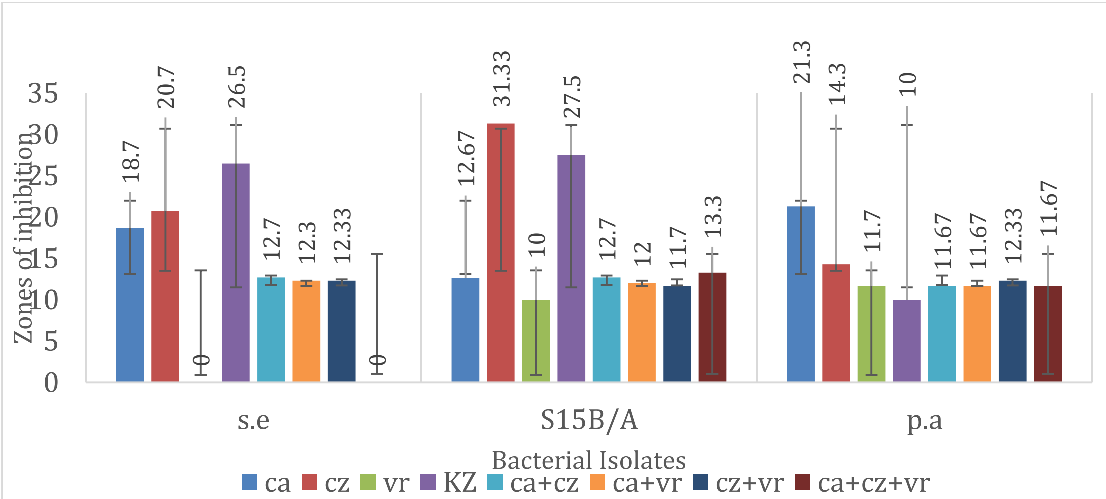

Figure 1. Mean values of zones of inhibition diameters (in mm) of plant extract activity individually and in combination against two *S. epidermidis* isolates and one *P. acnes* isolate.

Previous studies have documented antibacterial activity of phytochemicals and stated that different plants have different mode of action in inhibiting bacterial growth particularly the saponins, alkaloids, flavonoids and terpenoids (Barbieri et al., 2017). The presence of these phytochemicals, particularly, in the ethanolic extracts of *C. aromatic* and *C. zedoaria* could explain the ability of these extracts to inhibit bacterial growth. Furthermore, the varying types of phytochemicals in the three extracts seem to correlate with their antibacterial activity with *C. aromatica* being the most effective plant in this study and had the most antibacterial activity even on antibiotic resistant bacteria such as MRSA and MRSE. This study shows the potential of using *C. aromatic* to develop healthcare products such as facewash and soap as it seems to be able to inhibit the growth of most of the isolates tested, most of them having been isolated from skin samples. However, more bacterial isolates need to be tested to give a more valid conclusion.

Conclusion

In conclusion, the individual plant extract of *C. aromatica*, was found to have the most antibacterial activity compared to other individual and combinatorial of plant extracts against a majority of the skin pathogens tested. However, there were some bacterial isolates that showed resistance to this plant extract. In these cases, the antibacterial activity of combinatorial plant extracts were found to be more effective compared to activity of *C. aromatica* alone. The antibiotic susceptibility screening showed that all the bacteria isolates tested had different susceptibility patterns to the range of antibiotics tested. This is due to the genetic variants among the strains causing them to have different susceptibility patterns to the antibiotics tested. Perhaps, when comparing to the zones of inhibition produced by plant extracts against all the bacteria tested, *C. aromatica* can be formulated into an effective facial product to inhibit growth of gram-positive bacteria on human skin.

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