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Potential Application of *Murraya koenigii* Extract as Antibacterial Agent in Liquid Hand Soap Formulation

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Abstract

Here we present the efficacies of *Murraya koenigii* leaves extract as natural antibacterial agents by the ability to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. The antibacterial activities of formulated liquid hand soap with different concentrations (10, 30 and 50 mg/mL) of *M. koenigii* extracts were determined by paper disc diffusion method. *M. koenigii* leaves extract at 10 mg/mL concentration had the ability to inhibit the growth of both test microorganisms. Whereas, for liquid hand soap formulated with 10, 30 and 50 mg/mL *M. koenigii* extracts, inhibition zones were observed on *S. aureus* assay plates but not on *E. coli*. Hence, the results suggested that liquid hand soap containing *M. koenigii* extract had therapeutic potentials to prevent spread of skin diseases caused by *S. aureus*.

1. INTRODUCTION

The use of herbal medicinal products and supplements has increased tremendously over decades with people worldwide relying on them for some part of primary healthcare. Some of these products have shown promising potential on the efficacies and undoubtedly established. Consumers concern about products that contain chemical or synthetic ingredients which could be harmful to them, and choose natural ingredients in personal care product over those that contain chemicals. Therefore, there is a considerable interest to develop and screen potential plant origin as natural antimicrobials agents due to the increase concerns on chemical ingredients among consumer. More attention is given to plant derived compound due to its potential as antimicrobial agent in cosmetic as well as the food product. *M. koenigii* or commonly known as curry leaf is one of the example. It is a leafy vegetable generally used as natural flavouring agents with several important health benefits. It contains several medicinal properties such as antioxidant, antimicrobial, antifungal, anti inflammatory (Hanan et al., 2016). The plant has been in use long times ago in traditional medicine systems in many countries especially India. Studies proved the antibacterial properties in *M. koenigii* is due to the presence of phytochemical such as alkaloids, saponins, flavonoids, terpinoids and others (Baskaran et al., 2011). According to Phillip (2009), there are more than 700 antimicrobial products in the market and nearly 76% of them are liquid soaps, indicating that antimicrobial products are strongly accepted by consumers. Many consumers who are conscious about hygiene will look for an antibacterial label on products when buying liquid or solid soap for extra protection. This study aims to evaluate the efficacy and potential application of *M. koenigii* leaf as natural antimicrobial agent in liquid hand soap. The efficacy of the sample extract as a natural antibacterial agent was evaluated with the ability to inhibit the growth of common microorganisms such as *E. coli* and *S. aureus*.

2. MATERIALS AND METHODS

2.1. Sample preparation

M. koenigii fresh leaves were washed with distilled water to remove dirt and oven dry at 60 °C for 10 to 15 minutes. The samples were considered dry when a constant weight value is obtained. Dried samples were ground into powder to create better surface contact with extraction solvent (ethanol). 70 g of the ground sample was extracted with 500 mL of ethanol in Soxhlet apparatus at 80 °C for 10 hours and filtered through filter paper. The organic extract was concentrated using rotary evaporator at 45 °C for 30 minutes and kept at 4 °C for further analyses.

2.2. Preparation of liquid hand soap

Liquid hand soap base was prepared based on Loyola University of Chicago lab manual (2013). 200 mL of glycerine was heated to 60 °C for 48 minutes to ensure

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methanol is driven off. 5 g of potassium hydroxide (KOH) solution was dissolved in 50 mL distilled water and added to 25 g of coconut oil. The mixture was gently poured into hot glycerine and constantly stirred until dissolved. 5 g of citric acid was then added into the solution. The temperature of soap was maintained at 70 °C with constant stir for 20 minutes. The soap was cooled at room temperature and pH was adjusted to 7. The efficacy of *M. koenigii* extracts in liquid hand soap was evaluated by adding the crude extract at concentrations of 10, 30 and 50 mg/mL to the liquid hand soap base.

2.3. Antibacterial assay

The The antibacterial activities of *M. koenigii* leaves extract and *M. koenigii* liquid hand soap were tested against *S. aureus* and *E. coli* using paper disc diffusion assay. Bacterial cultures were freshly prepared by inoculating a single colony of test organism in triptic soy broth (TSB) at 37 °C for 24 hours. 0.2 mL of the bacterial inoculums was spread evenly on triptic soy agar (TSA) medium. 5 and 10 mg/mL of *M. koenigii* leaves extract and 10, 30 and 50 mg/mL of *M. koenigii* liquid hand soap, were pipetted onto a sterile 6 mm paper disc. Paper discs were dried in a laminar air-flow prior to transfer onto the TSA containing bacterial inoculums. Trimethoprim (50 µg/mL) was used as positive control, whereas sterile distilled water and liquid hand soap base were used as negative controls, respectively. Plates were incubated at 37 °C for 24 hours. Assays were carried out in triplicate. Presence or absence of inhibition zone was observed and recorded during the incubation period.

3. RESULTS AND DISCUSSION

The *M. koenigii* leaves were successfully extracted by Soxhlet extraction using ethanol as solvent. From 70 g of powdered samples, 12.4% crude extract was recovered. The extract was blackish green in colour and with a sticky texture. With Soxhlet extraction, the solvent can be recycled which directly can reduced the amount of ethanol used. However, there are numerous factors that can affect the extraction efficiency such as temperature, solvent sample ratio, type and concentration of solvent, size of plant materials, extraction time needed, agitation speed and extraction pH need to be considered for this method (Chirinos et al., 2007). The difference in solvent polarity is greatly influenced percentage of yield, phytochemical compounds and antimicrobial efficacy of plant extract. According to Nur Syukriah et al. (2014), generally polar solvent such as ethanol will produce higher extraction yield when compared to the non-polar solvent such as chloroform. In this study, ethanol was used to extract *M. koenigii* because it is harmless as compared to methanol. Previous studies by Hanan et al. (2016) and Prethyusha et al. (2016) reported that with ethanol as extraction solvent, they managed to get higher yield of *M. koenigii* crude extracts. Even though water is harmless and cost effective, it produced lower extraction yield as compared to ethanol with 17.7% and 20.6% respectively (Indu and Nirmala, 2011). Moreover, the boiling point of water comparably higher than ethanol, thus will require more time and higher temperature in getting the crude extracts.

The antibacterial activities of *M. koenigii* leaves were assessed according to the average diameter zone of inhibition against *E. coli* and *S. aureus* at different concentrations (5 and 10 mg/mL). *M. koenigii* leaves extract at concentration of 10 mg/mL showed antibacterial activity against both *E. coli* and *S. aureus* with inhibitory zones of 3.2 mm and 5.9 mm respectively (Figure 1). *S. aureus* had a bigger zone of inhibition as compared to *E. coli* (Table 1). Similar results were recorded by Hanan et al. (2016) and Prathyusha et al. (2016). Whereas, at concentration of 5 mg/mL, no inhibition zones were observed on both test microorganisms. This may due to the lower concentration of plant extract used. Moreover, secondary metabolites produced by plant are influenced by the soil and several environmental factors (Pavarini et al., 2012). Previous study by Hanan et al., 2016 indicates that minimum inhibitory concentration of *M. koenigii* extract to *E. coli* and *S. aureus* was at 6.25 mg/mL by ethanol extraction. Thus, 5 mg/mL concentration of plant extract was not enough to inhibit the growth of both test microorganisms. Selvamani and Balamurugan (2014) also reported that *M. koenigii* leaves extract had potent antibacterial activity against Gram-positive and Gram-negative bacteria. This indicates the presence of broad spectrum of antibacterial substances in *M. koenigii*.

Table 1: Average inhibition zone (mm) of *M. koenigii* leaves extract against test microorganisms

Test Microorganism	Concentration of *M. koenigii* leaves extract (mg/mL)		Positive control	Negative control
	5	10		
E. coli	-	3.20	18.50	-
S. aureus	-	5.90	17.55	-

Note: (-) indicates no zone of inhibition.

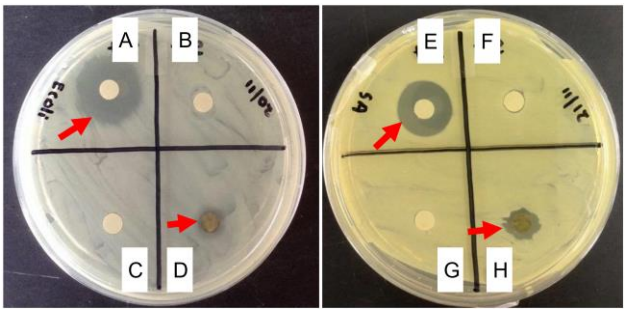

Figure 1: Inhibition zones (red arrow) observed on assay plates of *M. koenigii* leaves extract against *E. coli* (left) and *S. aureus* (right) at concentration of 10 mg/mL. A and E: Positive control, B and F: Negative control, C and G: 5 mg/mL *M. koenigii* leaves extract, D and H: 10 mg/mL *M. koenigii* leaves extract.

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The potential application of *M. koenigii* leaves extract in liquid hand soap was assessed at 3 different concentrations; 10, 30 and 50 mg/mL. Results showed that no inhibition zones were observed in all concentrations of liquid hand soap formulated with *M. koenigii* against *E. coli*. However, contrast results were observed against *S. aureus* at all concentrations with 1.3 mm, 5.6 mm and 8.9 mm inhibition zones recorded, respectively (Table 2). Thus, indicates that the efficacy of formulated liquid hand soap to inhibit the growth of *S. aureus* is increased with the increase of *M. koenigii* extract concentrations (Figure 2). *S. aureus* is known as causative bacteria for skin infections including boils, thrush, impetigo (Aliyu et al., 2012). Liquid hand soap base without addition of *M. koenigii* extract which served as negative control showed no inhibition zone in both tested microorganisms. This shows that plain liquid hand soap with no antibacterial agent added could not inhibit the growth of microorganism. However, adding too much of antibacterial agent may cause some safety concern such as antimicrobial resistance, more sensitive to allergies and skin rashes to human (Selvamohan and Sandhya, 2012).

Table 2: Average inhibition zone (mm) of *M. koenigii* liquid hand soap against test microorganisms

Test microorganism	Liquid hand soap with different concentration of *M. koenigii* leaves extract (mg/mL)			Positive control	Negative control
	10	30	50		
E. coli	-	-	-	18.30	-
S. aureus	1.3	5.60	8.90	17.00	-

Note: (-) indicates no zone of inhibition.

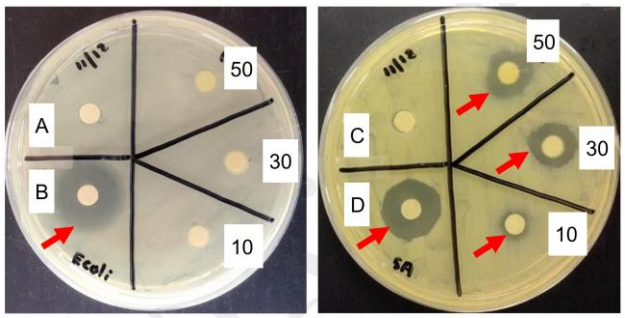

Figure 2: Inhibition zones (red arrow) observed on assay plates of liquid hand soap formulated with *M. koenigii* leaves extract against *E. coli* (left) and *S. aureus* (right). A and C: Negative control, B and D: Positive control, 10: liquid hand soap + 10 mg/mL *M. koenigii* leaves extract, 30: liquid hand soap + 30 mg/mL *M. koenigii* leaves extract, 50: liquid hand soap + 50 mg/mL *M. koenigii* leaves extract

Soap should contain good ingredients that have the ability to kill bacteria but not to damage human body tissues (Varsha, 2016). Therefore, an adequate amount of antibacterial agent can be added into liquid hand soap base in order to prevent the growth of the microorganism. In this study, no inhibition zones were observed on assay against *E. coli* may due to insufficient concentration of plant extract that used to prepare the liquid hand soap. The amount is not enough to inhibit the growth of bacterial as the antibacterial components in the extract might not be stable in the liquid soap medium. The antibacterial agents in plant extract could be degraded during liquid hand soap preparation process (Wijetunge and Perera, 2015). Besides, the inhibition zones observed on *S. aureus* assay plates were as expected due to the presence of outer membrane in Gram-negative *E. coli* that make it less susceptible to antimicrobials than Gram-positive bacteria (Vaara, 1992; Simões et al, 2008). The lipopolysaccharide in the outer membrane of Gram-negative functions as a barrier that slows the penetration of antimicrobials. The passage through the outer membrane of this bacteria is regulated by the presence of hydrophilic channels (porins) that usually exclude the entry of hydrophobic compounds (Bos, 2004; Cohen, 2011). This will exclude the antibiotics from entering the cell (Zaidan et al., 2005). The outer peptidoglycan layer of Gram-positive bacteria *S. aureus* is permeable to the antimicrobial chemical substances. Therefore, Gram-negative bacteria *E. coli* is less susceptible toward inhibition of the antibacterial agent that present in the solvent extracts (Nikaido, 2003). Bacteria also show different ways of motility. *E. coli* can swim and swarm using its two forms of surface flagella-dependent motility which is not exist in *S. aureus* (Harshey, 2003; Borges, 2012; Lemos, 2014;). These types of motility contribute to the virulence of pathogens through adhesion and biofilm formation on biotic and abiotic surfaces (Borges, 2012).

4. CONCLUSION

The present study proved that the antibacterial efficacy of *M. koenigii* extracts able to inhibit the growth of both *E. coli* and *S. aureus* at concentration of 10 mg/mL. However, liquid hand soap formulated with different concentrations (10, 30 and 50 mg/mL) of *M. koenigii* extract only able to inhibit the growth of *S. aureus*. The findings suggest that liquid hand soap containing *M. koenigii* extract had therapeutic potentials to treat diseases due to its susceptibility towards *S. aureus*. Hence, it can be used to prevent skin infections and transmission of skin pathogens caused by *S. aureus* when used in hand washing.

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