

## Antimicrobial Activity of Curry Leaves (*Murayya koenigii*) on Selected Foodborne Pathogens

Bazlee Abu Bakar\*, Wan Razarinah Wan Abdul Razak\*, Che Puteh Osman

Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

Corresponding author: [bazleeabubakar@gmail.com](mailto:bazleeabubakar@gmail.com) / [razarina408@uitm.edu.my](mailto:razarina408@uitm.edu.my)

Received: 15 May 2019; Accepted: 8 November 2019; Published: 31 January 2020

### ABSTRACT

Food poisoning is one of the most common diseases in the world in which are caused by ingesting foodborne pathogens. Study was conducted to determine the antimicrobial activity of *Murayya koenigii* leaves extract on foodborne pathogens namely *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The antimicrobial activity was determined at four different concentrations of 150 mg/mL, 200 mg/mL, 250 mg/mL and 300 mg/mL. *S. typhi* was the most sensitive to *M. koenigii* extract at concentration 300 mg/mL with zone of inhibition diameter mean value of  $8.67 \pm 0.67$  mm followed by *E. coli*, *S. aureus* and *B. cereus* with zone of inhibition diameter mean values of  $8.33 \pm 0.88$  mm,  $8.00 \pm 0$  mm and  $7.67 \pm 0.33$  mm. *C. albicans* and *A. niger* were resistant to the extract. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against *S. typhi*, *S. aureus*, *B. cereus* and *E. coli* were 75 mg/mL, 150 mg/mL, 125 mg/mL and 100 mg/mL respectively. There were no significant differences on the concentrations of *M. koenigii* extract on the zone of inhibition diameter produced ( $p=0.056$ ). *M. koenigii* leaves extract exhibit antibacterial activity but no antifungal activity.

**Keywords:** *Murayya koenigii*; Antibacterial; Antifungal

### INTRODUCTION

*Murayya koenigii*, commonly known as “Daun Kari” is a type of common aromatic plant that always being used in cooking for natural flavoring and aroma. One of the major problems that cause harm to human and food spoilages are the microorganism that are contaminated inside the food. The survival of the microbes in food is a crucial issue which can lead to the spoilage and reduce the quality of food products and also cause harm to human if they are being ingested [1]. Food poisoning is one of the most common diseases in the world. Food poisoning is usually caused by ingesting food containing microbes that can be harmful to humans. These microbes would usually produce toxic and also gas in which will give a bad side effect to humans. In South Korea, the approximate number of patients warded for foodborne diseases is estimated to be 20 times higher than the number of reported cases by the Ministry

of Health of South Korea [2]. It was reported that in Malaysia the cases of food poisoning are increasing by the rate of 62.47 cases in 100,000 people [3]. Many microbes are now resistant to the antibiotics and antifungals that are available in the market. In addition, the dosage of antibiotic and antifungal has been increased due to the resistance of the microbes. Therefore, people are looking for another alternative to replace antibiotics and antifungals in order to treat bacterial infections and also fungal infections in particular natural based products. This study aims to evaluate the antimicrobial activity of *M. koenigii* water based extract at different concentration on selected foodborne pathogens which were *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404.

Cellulose can be found in plant cell wall mostly that forming the structural fibre of plants and it gives strength because it is the most abundant organic compound, for example, stem, leaves, and branches. It is organized into microfibril in the cell wall, interrupted by hemicellulose and surrounded by a lignin matrix [1]. For example, cellulose can be extracted from potatoes, rice husk, wood pulps, durian rinds, and jackfruit rinds. Jackfruit rinds (JR) are always discarded since they are not edible but the rinds are a good source of cellulose and it can be extracted by using suitable extraction method.

Jackfruit or *Artocarpus heterophyllus* is occasionally found in Pacific island home gardens and commonly found in Southeast Asia. It is well known as an aromatic, juicy and flavourful fruit that can be eaten fresh or preserved. The fruit has many benefits from the seeds until the wood chips yield. For example, the seeds can be boiled, roasted, eaten like chestnut or cooked in other dishes, the wood chips can be used as a dye to give colour to the robes of Buddhist priest but the rind is always discarded [2]. The rind contained cellulose up until 27.75% depending on the type of extraction [3]. The aim of this study is to extract cellulose from JR by using bleaching treatment and alkaline treatment and to characterize the cellulose contents.

## EXPERIMENTAL

The methods that were used in this study are preparation of plant materials, preparation of extract, agar disc diffusion method and broth microdilution method. The agar disc diffusion method was used to determine the antibacterial activity while the microdilution method was used to identify the minimum inhibitory concentrations (MICs).

### Raw materials

The *M. koenigii* leaves were bought fresh from a wet market in Petaling Jaya, Selangor while the bacteria and fungi were obtained from the Microbiology lab at Faculty of Applied Sciences, UiTM Shah Alam.

### Preparation of plant material

The *M. koenigii* leaves were washed by using a tap water and were left air dried for two weeks. The dried *M. koenigii* leaves were then grinded by using a blender until it became powder form.

### **Preparation of extract**

An amount of 200 g of powdered *M. koenigii* leaves were soaked in 500 mL of cold distilled water for 5 days and kept inside the refrigerator. The soaked *M. koenigii* leaves were then boiled for 1 hour and the content was strained and filtered by using a filter paper (Whatman no.4) before putting inside a freezer for one day. The extract was then freeze dried by using a freeze dryer.

### **Preparation of inoculum**

An amount of 4.25 g of sodium chloride was mixed with 500 ml of distilled water in a Schott bottle and 10 mL of the saline solution was dispensed into the culture bottles. The culture bottles and its content were autoclaved for 121 °C for 15 minutes. This saline solution was used to subculture the microbes for the antimicrobial testing. An amount of 0.5 mL of the all the cultures were transferred into the saline solutions and were compared with 0.5 McFarland Standard (Hardy Diagnostics, United States).

### **Agar disc diffusion method**

This method was adopted from Yildirim *et al.*, (2017) [4] with slight modification. An amount of 0.5 mL of inoculum which was compared with McFarland standard ( $\pm 1.0 \times 10^8$  CFU) (Hardy Diagnostics, United States) were spread on the MHA (Mueller Hinton agar Oxoid CM0337, United Kingdom) plates by using a right angle glass spreader. An aseptic technique was carried out by flaming the forceps and the paper discs with 6mm diameter size (Oxoid Antimicrobial Susceptibility Test Disc, United Kingdom) were dipped into the *M. koenigii* leaves extract at four different concentrations which are 150 mg/mL, 200 mg/mL, 250 mg/mL and 300 mg/mL. The discs were placed on the agar plates. The agar plates were then incubated for 48 hours at 37 °C. The diameter of inhibition zones was measured in mm and compared. These steps were repeated by using the selected fungi in which the broth culture were spread on SDA (Sabouraud Dextrose Agar, Oxoid CM0041, United Kingdom) and the agar plates were incubated at 27 °C for 5 days [5]. The antibiotic Gentamicin and antifungal Nystatin were used as positive control while distilled water were used as negative control.

### **Broth microdilution method**

Broth microdilution method was used to determine the minimum inhibitory concentrations (MICs). An amount 0.5 mL of sterile MHB (Mueller Hinton Broth, SRL 49550 MM0210, India) was added to all the 8 well of microtiter plates (HmbG PO458 Microplate 96 wells Flat Bottom, Malaysia) by using the micropipette. An amount of 0.5 mL of the extract was added into the first well. An amount of 0.5 mL from the first well which contains extract and sterile MHB (Mueller Hinton Broth, SRL 49550 MM0210, India) was transferred to the second well. By using a separate micropipette tip, the content of the second well was mixed and 0.5 mL was transferred to the third well. This step was continued until the eighth well. In the eighth well, 0.5 mL of the content was removed so that the final volume in all tubes is 0.5 mL. Then, an amount of 0.2 mL of selected bacteria (compared with McFarland standard (Hardy Diagnostics, United States) was added to all of the wells. The microtiter plate was incubated for 48 hours at 37 °C. This method was repeated by using selected fungi in which the broth media used was Sabouraud dextrose broth (SDB, Difco™ Sabouraud Dextrose Broth 238230) and the microtiter plate was incubated at 27 °C for 5 days. The highest dilution without the growth was taken as the MIC [6].

## Determination of MBCs and MFCs

As for the minimum bactericidal concentration (MBC), all the dilutions without growth were subcultured to the MHA plates (Mueller Hinton agar Oxoid CM0337, United Kingdom). The MHA agar plates (Mueller Hinton agar Oxoid CM0337, United Kingdom) were incubated for 48 hours at 37 °C. The agar plate with lowest concentration that did not show any bacterial growth was taken as the MBC. As for the minimum fungicidal concentration (MFC), the steps were the same as the determination of MBC only the differences were the incubation temperature, media and incubation period which are at 27 °C for 5 days [7].

## Statistical analysis

The experiment was repeated three times in order to obtain the mean values (mean  $\pm$  SEM) and all of the data was analyzed by one-way ANOVA (IBM SPSS Statistic 22, 2013).

## RESULTS AND DISCUSSION

The result of the antimicrobial activity of *M. koenigii* water based extract was shown in the Table 1, at the concentration of 150 mg/mL, the extract was most effective against *S. aureus* in which the diameter means of inhibition zone produced was  $8.00 \pm 0.57$  mm in diameter followed by *S. typhi* with  $7.33 \pm 0.33$  mm. As for *B. cereus* and *E. coli* the inhibition zone produced was  $7.00 \pm 0$  mm in diameter. At the concentration of 200 mg/mL, the highest mean diameter of inhibition zone produced was against *S. aureus* measuring  $8.00 \pm 0.57$  mm in diameter followed by *E. coli*, *S. typhi* and *B. cereus* which the diameter produced were  $7.67 \pm 0.67$  mm,  $7.33 \pm 0.33$  mm and  $7.00 \pm 0$  mm respectively. At the concentration of 250 mg/mL, inhibition zones were observed against four selected foodborne pathogens which were  $8.00 \pm 0.57$  mm and  $8.00 \pm 0$  mm in diameter against *E. coli* and *S. aureus*,  $7.67 \pm 0.33$  mm in diameter against *S. typhi* and  $7.33 \pm 0.33$  mm against *B. cereus*. At the concentration of 300 mg/mL, inhibition zones were observed at  $8.67 \pm 0.67$  mm in diameter on *S. typhium*,  $8.33 \pm 0.88$  mm in diameter on *E. coli*,  $8.00 \pm 0$  mm in diameter on *S. aureus* and  $7.67 \pm 0.33$  mm on *B. cereus*. As shown in Table 2, there were no inhibition zones produced against *C. albicans* and *A. niger* at all four concentrations. There were no significant differences  $p > 0.05$  ( $p$  value = 0.056) in the zone of inhibition diameter mean values between the selected bacteria when tested against this extract. The minimum inhibitory concentration (MIC) of *M. koenigii* against *S. typhium* was at 75 mg/mL while for *S. aureus*, it is at 100 mg/mL. As for *B. cereus* and *E. coli* the MIC were determined at 125 mg/mL and 150 mg/mL respectively. However, the minimum bactericidal concentrations (MBCs) were the same as the minimum inhibitory concentrations (MICs) for all the tested bacteria.

Overall, in this research, the extract was the most effective against *S. typhi* whereby the highest values of diameter inhibition zones were observed against these bacteria and the MIC and the MBC values were the lowest specifically on these bacteria compared to the other bacteria. Furthermore, this showed that *M. koenigii* leaves extract exhibited weak antibacterial activity whereby the diameter of clear zones produced were less than 10 mm. There were no significant differences on the concentrations of *M. koenigii* extract on the zone of inhibition diameter produced where the  $p$  value is 0.056. This study was supported by [8] that stated the zinc oxide nanoparticles obtained from curry leaves were successful against *S. aureus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, *C. albicans* and *C. tropicalis*. This shows that the

contents inside the *M. koenigii* itself have strong antimicrobial activity when it was isolated. In comparison between *M. koenigii* extract and zinc oxide nanoparticles against *C. albicans*, zinc oxide nanoparticles were effective against *C. albicans* compared to *M. koenigii* extract. The methanol and ethanol extract of *M. koenigii* against (gram positive) and (gram negative bacteria) namely *S. aureus*, *Streptococcus* and *E. coli* were potent but unfortunately ineffective against *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [9]. Another evidence that shows curry leaves have strong antibacterial properties are the monomeric protein or antioxidant protein (APC) isolated from the curry leaves shows an antimicrobial activity against *S. aureus*, *Vibrio cholera*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *S. typhium* [10]. In previous research that was conducted by Dodanna *et al.*[11], the aqueous extract of *M. koenigii* did not produce any inhibition zones on *C. albicans* while the 100% ethyl alcohol extract of *M. koenigii* produced clear zones on *C. albicans* with inhibition zones mean value of  $24.05 \pm 0.07$  mm. Researchers have proposed that the compounds that were identified in the plant extracts namely alkaloid, phenolic compounds are associated with the extracts antimicrobial characteristics in which these compounds causes the disturbance of the outer part of the bacterial cell that causes cell death [12-14].

**Table 1:** Mean  $\pm$  SEM diameter of inhibition zone (mm) of *M. koenigii* water based extract against selected foodborne bacteria

Foodborne bacteria	Concentration of extract (mg/mL)				Gentamicin (+)	Distilled water (-)
	150	200	250	300		
<i>S. typhi</i> ATCC 14028	$7.33 \pm 0.33$	$7.33 \pm 0.33$	$7.67 \pm 0.33$	$8.67 \pm 0.67$	16	NIZ
<i>S. aureus</i> ATCC 25923	$8.00 \pm 0.57$	$8.00 \pm 0.57$	$8.00 \pm 0$	$8.00 \pm 0$	20	NIZ
<i>B. cereus</i> ATCC 11778	$7.00 \pm 0$	$7.00 \pm 0$	$7.33 \pm 0.33$	$7.67 \pm 0.33$	21	NIZ
<i>E. coli</i> ATCC 25922	$7.00 \pm 0$	$7.67 \pm 0.67$	$8.00 \pm 0.57$	$8.33 \pm 0.88$	17	NIZ

NIZ: No inhibition zone

**Table 2:** Mean  $\pm$  SEM of diameters of inhibition zones (mm) of *M.koenigii* extract on two selected fungi

Fungi	Concentration of extract (mg/mL)				Nystatin (+)	Distilled water (-)
	150	200	250	300		
<i>A.niger</i> ATCC 16404	NIZ	NIZ	NIZ	NIZ	18	NIZ
<i>C. albicans</i> ATCC 10231	NIZ	NIZ	NIZ	NIZ	25	NIZ

NIZ: No inhibition zone

**Table 3:** Concentration of MICs and MBCs of *M. koenigii* extract on selected foodborne pathogens

Foodborne pathogens	MIC (mg/mL)	MBC (mg/mL)
<i>S. typhi</i> ATCC 14028	75	75
<i>S. aureus</i> ATCC 25923	100	100
<i>B. cereus</i> ATCC 11778	125	125
<i>E. coli</i> ATCC 25922	150	150

## CONCLUSIONS

As a conclusion, this research shows that *M. koenigii* extract exhibit antibacterial properties against selected foodborne pathogens.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge Universiti Teknologi MARA in providing the GIP research grant project (Project Number:600-IRMI 5/3/GIP (026/2019)) and Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.



## REFERENCES

- [1] Celiktas, O.Y., Bedir, E., Sukan, F.V., *In vitro* antioxidant of *Rosamrinus officinalis* extracts treated with supercritical carbon dioxide, *Food Chemistry*, **101**: 1457-1464 (2007).
- [2] Kim, Y. S., Lee, S. H., Kim, S. H., Joo, Y., Bahk, G. J., Investigation of the experience of foodborne illness and estimation of the incidence of foodborne disease in South Korea, *Food Control*, **47**: 226-230 (2015).
- [3] Soon, J. M., Singh, H., Baines, R., Foodborne diseases in Malaysia: A review, *Food Control*, **22**(6): 823-830 (2011).
- [4] Yildirim, S. T., Oztop, M. H., Soyer, Y., Cinnamon oil nanoemulsions by spontaneous emulsification: Formulation, characterization and antimicrobial activity, *LWT - Food Science and Technology*, **84**: 122-128 (2017).
- [5] Xie, Y., Wang, Z., Huang, Q., Zhang, D., Antifungal activity of several essential oils and major components against wood-rot fungi, *Industrial Crops and Products*, **108**: 278-285(2017).
- [6] Hastey, C. J., Dale, S. E., Nary, J., Citron, D., Law, J. H., Roe-Carpenter, D. E., Chesnel, L., Comparison of *Clostridium difficile* minimum inhibitory concentrations obtained using agar dilution vs broth microdilution methods, *Anaerobe*, **44**: 73-77(2017).
- [7] Chen, Y.-W., Ye, S.-R., Ting, C., Yu, Y.-H., Antibacterial activity of propolins from Taiwanese green propolis, *Journal of Food and Drug Analysis*, 1-8 (2017)
- [8] Elumalai, K., Velmurugan, S., Ravi, S., Kathiravan, V., & Ashokkumar, S., Bio-fabrication of zinc oxide nanoparticles using leaf extract of curry (*Murayya koeniggi*) and its antimicrobial activity, *Material Science in Semiconductor Processing*, **34**: 365-372 (2015).
- [9] Al Harbi, H., M.Irfan, U., Ali, S., The antibacterial effect of curry leaves (*Murayya koenigii*), *European Journal of Pharmaceutical and Medical Research*, **3**(10): 382-387(2016).
- [10] Nigappa, M.B., Dhanajaya, B.L., Dinesha, R., Harsha, R., Srinivas, L., Potent antibacterial property of APC protein from curry leaves (*Murayya koenigii* leaves), *Food Chemistry*, **2**(5): 221-227 (2009).
- [11] Doddanna, J. S., Patei, S., Sundarrao, A. M., Veerabhadrapa, S. R., Antimicrobial activity of plant extracts on *Candida albicans* : An *in vitro* study, *International Journal of Dental Research*, **24**(4): 401- 405 (2013).
- [12] Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., Bakri, M. M., Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, *Saudi Journal of Biological Sciences*, **25**(2): 361-366 (2018).
- [13] Burt, S., Essential oils: their antibacterial properties and potential application in foods, *International Journal of Food Microbiology*, **94**: 223- 253 (2004).
- [14] Gill, A.O., Holley, R. A., Disruption of *Escherichia coli*, *Listeria monocytogens* and *Lactobacillus sakei* cellular membranes by plant aromatic oils, *International Journal of Food Microbiology*, **108**: 1-9 (2006)