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Nurul Fadzlin Ab Llah, Khomaizon Abdul Kadir Pahirulzaman, Nurul Amira Zainurin, et al.

Antimicrobial Activities and Total Yield of Potential Natural Preservatives Extracted using Different Solvents

Nurul Fadzlin Ab Llah[1, a)], Khomaizon Abdul Kadir Pahirulzaman[1, b)], Nurul Amira Zainurin[1, c)], Maryana Mohamad Nor[1, 2, d)], Seri Intan Mokhtar[1, e)], Noor Hafizoh Saidan[1, f)]

[1]*Faculty of Agro Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia*
[2]*Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia*

[a)]f20d001f@siswa.umk.edu.my
[b)]khomaizon@umk.edu.my
[c)]f20d0242f@siswa.umk.edu.my
[d)]maryana.mn@umk.edu.my
[e)]intan@umk.edu.my
[f)]Corresponding author: hafizoh.s@umk.edu.my

Abstract. Traditionally, natural preservatives are added to the palm saps to avoid spontaneous fermentation. Three selected plants have been used, namely chengal wood chips (*Neobalanocarpus heimii),* mangosteen rind (*Garcinia mangostana* Linn.), and guava leaves (*Psidium guajava* Linn.). The present study aims to estimate the total yield of potential natural preservatives extracted using different solvents and investigate their antimicrobial activity. Chengal wood chips, mangosteen rind, and guava leaves were extracted with methanol, ethanol, and water by the hot maceration technique. The total yield for each extract (methanol, ethanol, and water) of chengal wood chips is 17.5 %, 14.04 %, and 16.46 %, respectively. The total yield for methanol, ethanol, and water extracts of mangosteen rinds is 22.58 %, 17.76 %, and 20.10 %, respectively. Besides, the total yield for methanol, ethanol, and water extracts of guava leaves are 16.64 %, 15.20 %, and 16.12%, respectively. The result has indicated that the best solvent to obtain the highest yield of extracts is the methanol solvent. Antimicrobial test of the potential natural preservatives was performed against *Escherichia coli, Streptococcus aureus,* and *Candida albicans.* Based on the results, none of the chengal and guava extracts can inhibit *E. coli* growth with 100 mg/mL concentration. Only mangosteen rind water extract can inhibit the *E. coli* growth with a 12.0±2 mm inhibition zone. For *S. aureus* inhibition, chengal wood chips extracts (methanol; 17±1.00 mm; ethanol, 17±0.00 mm; water, 17.33±0.58 mm) give the best inhibition zone slight difference between the solvents. The result for *S. aureus* inhibition is followed by mangosteen rind extracts (methanol, 11.79±0.29 mm; ethanol, 10.5±0.50 mm; water; 11±1.00 mm) and guava leaves extract (methanol, 12±0 mm; ethanol, 11.33±0.58 mm; water, 10±0 mm). For *C. albicans* inhibition, none of the extracts has shown antimicrobial activity. In conclusion, the results have indicated that different extracts have had different antimicrobial activity against the selected microbes due to the different active compounds in the extracts.

INTRODUCTION

Food spoilage caused by microorganisms is an immense concern for the food industry. At least 25% of all food produced is lost after harvesting due to microbial spoilage. The growth of spoilage microbiota in foods is not harmful to human health. However, it may alter the shelf-life, textural characteristics, and overall quality of the finished products, which will further cause commercial losses. Thus, it is essential to inhibit or reduce the growth of microorganisms in food products. The most widely used method in controlling microbial growth is using chemical additives. However, there are concerns about the safety and impact on human health, leading to increased demand for

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natural compounds as food preservatives. The antimicrobial properties of natural substances such as plant essential oils and extracts have been extensively studied with promising results [1].

Preservatives were used to preserve products from immediate damage after the manufacturing process. The factors contributing to the quality loss of food products are physical, chemical, microbiological, and enzymatic changes [2]. This study has used chengal wood, guava leaves, and mangosteen rind as potential natural preservatives for saps. Different solvents were used to extract compounds from the potential natural preservatives. Antimicrobial activities of the crude extracts were determined against *E. coli*, *S. aureus* and *C. albicans*. These potential natural preservatives will be used in coconut saps production to prevent or delay the fermentation process. Palm saps are categorized as beverages that can easily be fermented over time. Over-ferment of palm saps may promote several diseases, including hernia, diarrhoea, and headache [3]. This is due to the fermentation process that is dominated by yeast and lactic acid bacteria [4].

MATERIAL AND METHODS

Preparation of Potential Natural Preservatives

Three plant samples were used in this study as potential natural preservatives; chengal wood, guava leaves and mangosteen rind. The chengal woods were obtained from a wood factory in Jeli, Kelantan, Malaysia. It was chopped into small pieces before ground into powder using a heavy-duty blender (Milux, Italy). The guava leaves were collected at Kampung Baru Sri Panji, Kuala Krai, Kelantan, Malaysia, whereas fresh and ripened mangosteen rind was bought from the fresh market in Jeli, Kelantan, Malaysia. The guava leaves and mangosteen rinds were rinsed with tap water to remove the dirt before being dried at 35 - 40 ºC and 50 - 55 ºC, respectively. The samples were considered fully dried once they reached constant weight. The dried leaves were ground into powder using a blender, whereas the dried mangosteen rinds were ground into powder using mortar and pestle. The samples were kept in a zip-lock bag at room temperature for future use.

Compounds Extraction from Potential Natural Preservatives

Compounds were extracted from powdered potential natural preservative samples using the hot maceration method with water, ethanol and methanol as solvent. The ratio of samples and solvent was standardized to 1:10 (w/v). Samples were soaked in the solvents at 55 ºC for 2 hours [5]. Mixtures were filtered through Whatman No. 1 filter paper and the crude extracts were dried using a rotary evaporator (Büchi, Switzerland) at different temperatures; water extract (60 ℃), ethanolic and methanolic extract (40 ℃) [6]. The dried extract was weighed and kept in a glass container for future use. The percentage yield of each crude extract was calculated using the formula (W2 - W1/W0) × 100, where W2 is the weight of crude extract after removal of solvent, W1 is the weight of crude extract with solvent, and W0 is the initial weight of powder sample.

Antimicrobial Analyses

The antimicrobial activities of chengal wood, guava leaves, and mangosteen rind crude extracts were tested against Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and mould *Candida albicans* using disc diffusion assay according to Kirby and Bauer method [7]. Test microbial plates were prepared as follows; *S. aureus* and *E. coli* starter cultures were grown in Nutrient Broth at 37 °C for 24 hours and inoculated on the Mueller Hinton agar using a sterile cotton swab. *C. albicans* was grown in Sabouraud Dextrose Broth at 28 °C for 48 hours and inoculated on the Sabouraud Dextrose Agar (SDA) using a sterile cotton swab. 6 mm diameter sterile paper discs were impregnated with 100 mg/mL of the crude extracts. The discs were then air-dried in laminar flow for few minutes before transferred onto the inoculated test microbial plates using sterile forceps. Positive controls: Trimethoprim, Sigma-Aldrich (50 mg/mL) was used for *S. aureus* and *E. coli,* and fluconazole, Sigma-Aldrich (150 mg/mL) for *C. albicans* assay plates, respectively. At the same time, DMSO is the negative control. Bacterial assay plates were incubated at 37 °C overnight, whereas *C. albicans* assay plates were incubated at 25 and 28 °C for 2 days. The formations of inhibition zones were observed daily and recorded. All tests were conducted in triplicate.

RESULTS AND DISCUSSION

Crude Extracts Total Yield

In this study, three solvents (water, ethanol and methanol) were used to extract compounds from the potential natural preservative samples. Mangosteen rinds extracted in all solvents show a higher total yield compared to chengal wood and guava leaves (Table 1). The highest percentage yield is methanolic mangosteen rind extract at 22.58%. A similar total yield was recorded from chengal wood (16.46%) and guava leaves (16.12%) crude extracts. Potential natural preservatives extracted using methanol as solvent have shown a higher total yield than when extracted using water and ethanol. The variation in extraction yields might be linked to the different chemical nature of the extractable compounds as well as the polarity of the solvent used. Several factors contribute to the extraction efficiency: the solvent used, type of extraction method, sample particle size, chemical nature of the phytochemical, and interfering substances during the extraction process [8]. The yield of extraction can also rely on the polarity of the solvent and composition of the sample with the same extraction time and temperature [9]. Water, ethanol and methanol were selected as extraction solvents based on the solubility of the target compound in the natural preservative samples. A higher yield of extracts was observed when methanol used in the extraction process. This might be due to the high polarity of the solvents [10]. The most important task in solvent extraction is to find a suitable solvent that can extract the target compound from the plant material. Thus, solvent or solubility screening through a prediction method or experiment-based is necessary [1]. The result also shows that all samples extracted with water produced a higher yield of crude extracts than when extracted using ethanol. This might occur due to the solvent polarity towards the compounds that increases from less polar to more polar. Apart from that, the yield of methanol extract (chengal; 17.5%, mangosteen; 22.58 %; guava, 16.64 %) is only slightly less than the water extract (chengal; 16.46%, mangosteen; 20.1 %, guava; 16.12%). This result may be derivative to the implicit solubility of proteins and carbohydrates in methanol and water than in ethanol [11].

TABLE 1. The percentage yield of potential natural preservatives crude extracts

Potential natural preservative crude extract	Percentage Yield (%) / Solvent		
	Water	Ethanol	Methanol
Chengal wood	16.46	14.04	17.50
Guava leaves	16.12	15.20	16.64
Mangosteen rind	20.1	17.76	22.58

Antimicrobial Analysis on Natural Preservatives Crude Extracts

The natural preservative crude extracts show high to low efficacy against *S. aureus* when extracted using water, ethanol and methanol (Table 2). However, these crude extracts do not indicate any activity on *C. albicans*. Only mangosteen rind water extract can inhibit the growth of *E. coli* (12.00 ± 2.00 mm). This is probably due to the presence of antimicrobial compounds in mangosteen water extracts to inhibit *E. coli* [12]. The largest inhibition zone (17.33 mm) was observed when chengal wood water extract tested on *S. aureus* compared to guava leaves, and mangosteen rinds water extract. Chengal wood chips can inhibit microorganisms such as *B. subtilis* and *S. aureus* [13]. The different solvents used to extract chengal wood resulted in a slight difference for the inhibitory zones formation of the *S. aureus*. Apart from that, guava leaves extracts (water, ethanol and methanol) only can inhibit *S. aureus*. This result is supported by the finding of [14] which reported that the extract has antimicrobial activity against *S. aureus* but non-effective on *E. coli*. In guava leaves composition, tannin is the compound that is responsible for antimicrobial activity [15]. The methanolic guava leaves extract shows a larger diameter of inhibition zone than ethanolic and guava leaves water extracts. This result can be correlated to the solubility of the tannin compounds in different solvents showing different inhibition zones against the *S. aureus*. For mangosteen rind extracts, the result shows that the extracts can inhibit *S. aureus* and *E. coli*. This result may be also due to the different solubility of the active compound in the different solvents. Within the mangosteen pericarp rind, xanthone compound was especially responsible for the antimicrobial activity [16]. The efficacy of the solvents is very crucial to extract this compound. After all, mangosteen rind extract results showed that methanolic mangosteen rind extract is effective against *S. aureus* whereas mangosteen rind water extract is effective against *E. coli*. Overall, chengal extracts (water, ethanol, and methanol) are more effective compared to guava leaves and mangosteen rind extracts (water, ethanol, and methanol) in inhibiting the gram-positive bacteria *S. aureus*.

TABLE 2. Average zone of inhibition (mm) of potential natural preservatives crude extracts against test microorganisms using paper disc diffusion method. (-) indicates no inhibition zone, NA indicates not applicable

Potential natural preservatives crude extract	Solvent	Diameter zone of inhibition (mm)		
		E. coli	*S. aureus*	*C. albicans*
Chengal wood	Water	-	17.33 ± 0.58	-
	Ethanol	-	17.00 ± 0.00	-
	Methanol	-	17.00 ± 1.00	-
Guava leaves	Water	-	10.00 ± 0.00	-
	Ethanol	-	11.33 ± 0.58	-
	Methanol	-	12.00 ± 0.00	-
Mangosteen rind	Water	12.00 ± 2.00	11.00 ± 1.00	-
	Ethanol	-	10.50 ± 0.50	-
	Methanol	-	11.17 ± 0.29	-
Negative control	NA	-	-	-
Positive control	NA	32.23 ± 0.90	31.45 ± 0.85	35.7 ± 0.47

CONCLUSION

To conclude, the highest yield of the extract depends on the polarity and composition of the sample. However, in this study, methanol extract shows the highest extraction yield compared to ethanol and water extracts. Different types of extract show different antimicrobial activity for microbiological analysis, probably because of the different compounds present in the extract. In this work, chengal extracts show a good inhibition zone against *S. aureus* compared to the other extracts. Besides, for *E. coli*, only mangosteen water extract shows an inhibition zone. For *C. albicans*, none of the extracts can inhibit the growth of the microbes due to the use of a low extracts concentration. *C. albicans* is the mould species type and may require a higher concentration of extract than other bacterial species to form a clear zone of the microbial colony.

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