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Evaluation of Phenolic Content and Antibacterial Activity of Coconut (*Cocos nucifera* L.) Shell and Coir Powder in Different Extraction Solvents

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ABSTRACT

The issue of microbial resistance to synthetic antibiotics is receiving more serious attention. There are many studies on the search for new sources of antibiotics or antimicrobials from natural sources. Coconut is one of the most important crops in tropical countries with various uses. Every part of the tree and coconut fruit is very useful with different purposes such as being processed into drinks, food, fibre, building materials, chemicals and medical agents. Studies have showed that coconut shell and coir have phenolic content that gives antioxidant and antimicrobial properties. However, studies like this for Malaysian coconut are very limited. This research activity was conducted to determine the antibacterial activity of coconut shell and coir. Coconut shells and coirs randomly obtained from MARDI Station Bagan Datuk, were dried and ground into powder, and extracted using different solvents namely ethanol, methanol and acetone. The antibacterial activity of shell and coir extracts was determined using disc diffusion method against five pathogenic strains which were *Bacillus cereus* ATCC 10876, *Cronobacter sakazakii* ATCC 29544, *Enterobacter aerugenes* ATCC 13048, *Listeria innocua* ATCC 33090 and *Staphylococcus aureus* ATCC 25923. The analysis results obtained showed that coconut shell and coir extracts have potential antibacterial activity against all pathogenic strains tested with a wide range of inhibition zone sizes starting from 7.75 to 11.25 mm for the shell extract and 9.25 to 17.00 mm for the coir extract. The usage of different solvents in extraction affected the phenolic content and antibacterial activity of coir and shell extracts. The coir showed higher ($p<0.05$) total phenolic content in all solvents compared to the shell. The highest antibacterial activity was observed in the ethanolic coir extract against *S. aureus*, while the lowest activity was against *C. sakazakii* in the ethanolic shell extract. Methanolic and acetone extracts for both shell and coir exhibited lower minimum inhibitory and minimum bactericidal concentrations compared to ethanolic extract. With the results of this study, it is hoped that coconut coir and shell can be studied further and processed into promising antimicrobial agents.

Keywords: Antibacterial, coconut, phenolic, solvent.

INTRODUCTION

Many studies have been done in searching for new antimicrobial agents or antibiotics especially from natural sources such as plants and crops. Fruit wastes such as mango (*Mangifera indica* L.) kernel, and herbs including holy basil leaves (Subbiya et al., 2013), kesum (*Persicaria minor*) (Musa Ahmed et al., 2015) and black turmeric (*Curcuma caesia*) are among natural sources shown to possess antimicrobial property that can be further developed into new natural drugs. These drugs may replace synthetic antibiotics to fulfil the increasing consumer awareness and preference for natural medicine. In addition, the increasing cases of microbial resistance to synthetic antibiotics due to their excessive use has also led to the study of antimicrobials from natural sources. Antibiotic-resistant bacteria pose a serious problem in the medical field

as it leads to the difficulties in treating diseases caused by microorganisms. Some bacteria have developed resistance to antibiotics that were once commonly used to treat them. For example, *Staphylococcus aureus* ('golden staph' or MRSA) and *Neisseria gonorrhoeae* are now almost always resistant to benzyl penicillin. Benzyl penicillin also has little effect on most microorganisms found in the human digestive gut (Better Health Channel, 2021). The World Health Organization (WHO) has recommended the use of traditional medicine from natural sources for medicinal purposes and the production of medicines (Mushore et al., 2013). While the U.S Food and Drug Administrator (FDA) has allowed the use of natural substances especially from trees or plants such as herbs in traditional medicine and food processing (Kraisintu, 2003).

Coconut (*Cocos nucifera* L.) is a plant species from the Arecaceae family that is one of the most important and native plants, especially in Southeast Asian countries. Each part of the coconut tree and its fruit has usage such as in the production of food, drink, building materials, kitchen-wares, crafts, fibre, activated charcoal, chemicals and for traditional therapy (Shettigar et al., 2014). About 60% of domestic agricultural waste is from coir and coconut shells (Rodrigues and Pinto, 2007). The coconut fruit wall consists of three layers which are the outer epicarp (skin), mesocarp (fibrous husk and coir) and endocarp (hard shell). Previous research reported that husk fibre from the shell and coir possessed antibacterial, antiviral, antifungal, antileishmanial and antioxidant activities (Heenataj et al., 2017). *In vitro* antibacterial testing of coconut husk crude extracts have been performed against 24 species of common food-borne pathogens (Akinyele et al., 2011). MATAG is the new hybrid of Malaysian coconut. MATAG is the nickname of a new hybrid coconut as a result of crossing the original coconut from Malaysia which is Malaysia Yellow Dwarf (MYD) or Malaysia Red Dwarf (MRD) as female parent with Tagnanan coconut as male parent from the Philippines. This hybrid produces high yield and early in maturity. MATAG was noted for its high yield of copra per hectare, easier to de-husk, larger nut size with higher water content and reduced husk thickness (Sharma et al., 2000).

Phenolic compounds and bioactive substances in plants are components that contribute to biological activities in plant cells such as antioxidant and antimicrobial activities. Previous studies mentioned the content of phytochemical compounds in coir and coconut shell such as tannins, saponins, steroids, lignin, and saturated and unsaturated fatty acids (Khalid Thebo et al., 2016; Mazaya et al., 2020), pentosans, cellulose, catechin and epicathecin (Heenataj et al., 2017). These compounds contribute to biological activities such as antioxidant, antimicrobial, antiviral, antileishmanial and cytotoxic properties. Catechin possesses cellular growth inhibitory property, thus contributes to anticancer, antimicrobial, antimutagenic and anti-inflammatory properties. Previous studies have shown that coconut coir and shell have antimicrobial properties. Mazaya et al. (2020) reported antimicrobial property of both young and old coconut shell extracts against *Acetobacter aceti* and yeast *Saccharomyces cerevisiae*. In addition, Cyriac et al. (2013) and Shettigar et al. (2014) reported antimicrobial activity in coconut husk (coir) against selected cariogenic bacteria and food pathogens, respectively. However, studies on the antimicrobial property of both coconut shell and coir are still limited and need further study especially in new coconut hybrids that have been and are being introduced. Therefore, this study aimed to screen the antimicrobial characteristics of Malaysian hybrid MATAG coconut coir and shell.

MATERIALS AND METHODS

Collection and Preparation of Sample Material

Coconut shells and coir were obtained from Malaysian Agricultural Research and Development Institute (MARDI) Station in Bagan Datuk, Perak, Malaysia. The samples were randomly selected, separated and dried under sunlight for 3 days. The dried shells and coir were ground using an industrial grinder until they become powder and stored in a container at room temperature (25 - 27°C). Sample preparation was done in 3 replicates.

Preparation of Crude Extracts from Coconut Shell and Coir Powder Using Different Extraction Solvents and Times

A 10 g of coconut shell and coir powders were weighed into a 250 mL round bottom flask before each flask was added with 200 mL of different solvents namely ethanol (Merck Milipore), methanol (Merck Milipore) and acetone (Merck Milipore). All samples were heated until boiled under a reflux for 1, 8 and 24 h. After each boiling time duration, the solution was filtered using a Whatman paper No. 4. Each extract was dried under a vacuum at 60 °C using a Rotary evaporator (Buchi, R-205) for about 40 min until it dried. The dried extracts were weighed and diluted with dimethyl sulfoxide (DMSO) solution and the mixtures were mixed using a sonicator (JAC ultrasonic, 1505) to achieve a final concentration of 100 mg/mL. Finally, the mixtures were transferred into 20 mL vials and kept at 5 °C for further analysis.

Total Phenolic Screening

The total phenolic content of the test samples was estimated according to the Folin–Ciocalteu colorimetric method as described by Mirfat et al. (2020). An amount of 50 µL test sample was mixed with 100 µL Folin Ciocalteu's phenol reagent. After 3 min, 100 µL 10% sodium carbonate (Na_2CO_3) was added to the reaction mixture and allowed to stand in the dark for 60 min. The analysis was carried out in triplicates with a minimum exposure of light. The resulting blue-coloured complex was measured at 725 nm absorbance against a blank using spectrophotometer. Gallic acid was used as a reference standard and the content of total phenol was expressed in gallic acid equivalents (GAE) in milligram per g samples using the calibration curve.

Antibacterial Screening

The antibacterial activity of coir and shell ethanolic extracts from the 24 h extraction was determined using disc diffusion method according to the Bauer et al. (1966) methods against five pathogenic bacterial strains, namely *Bacillus cereus* ATCC 10876, *Cronobacter sakazakii* ATCC 29544, *Enterobacter aerugenes* ATCC 13048, *Listeria innocua* ATCC 33090 and *Staphylococcus aureus* ATCC 25923. All strains were grown in nutrient broth overnight before being diluted using Ringer's solution to a concentration of 1 x 10^8 CFU/mL equivalent to a 0.5 McFarland standard turbidity. An amount of 100 µL of the strain inoculum was pipetted and spread on solidified Mueller Hinton agar (Oxoid) in a petri dish using a sterile cotton swab before placing a sterile diffusion disc. A total of 20 µL of the extract solution at final concentration 100 mg/mL was pipetted onto the disc. Penicillin disc (10 µg) (Oxoid) was used as a control. All petri dishes were incubated in an incubator for 24 h at 37 °C. Any formation of a clear zone around the diffusion disc was measured and recorded in mm.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for all extracts were determined using microdilution method according to Musa Ahmed et al. (2015). The extracts at 100 mg/mL were subjected to two-fold serial dilution, to obtain a range of concentrations of 0.19 to 100 mg/mL. An aliquot of 100 µL of previously prepared standardised bacterial inoculum was added into 96-well microplate, before being mixed with 100 µL of prepared diluted extract. The microplate was then incubated at 37 °C overnight. The wells were then observed for the presence or absence of visible turbidity. The lowest concentration that showed no turbidity (no growth of bacteria) was reported as MIC. An amount of 100 µL from each well of each sample were pipetted onto solidified Mueller Hinton agar and spread using sterile cotton swab. The media were incubated at 37 °C for 24 h. The lowest concentration that did not show bacterial growth was considered as MBC.

Statistical Analysis

Data from three replicates of each sample were used for statistical analysis. The data analysis was done using Analysis of Variance (ANOVA) IBM SPSS software version 26. Mean comparison between samples and treatments was obtained using Duncan's multiple range test at $p < 0.05$ level.

RESULTS AND DISCUSSION

The phenolic content in shell powder and coir extract is shown in Table 1. Data were compared between coconut shell and coir in three different solvents and extraction times. Data were presented as mean of three replicates. Mean followed by different first letter indicates significant difference between extracts, while mean followed by different second letter indicate significant difference between extraction times. For shell powder, it was found that extraction using ethanol showed the highest ($p<0.05$) total phenolic content in all three extraction times (1, 8 and 24 h) compared to methanol and acetone extracts. However, in contrast to shell powder, the methanolic and acetone extracts of coir powder recorded significantly higher ($p<0.05$) total phenolic content than the ethanolic extract in all three extraction times. Total phenolic content was significantly higher in coir ($p<0.05$) where methanol and acetone extract showed the highest, compared to all shell extracts. All three coir extracts showed the highest ($p<0.05$) phenolic content in 8 h extraction compared to 1 and 24 h. As for shell powder, the ethanol extract did not show a significant difference ($p>0.05$) in phenolic content between extraction times, but the 24 h extraction in methanol and acetone recorded higher ($p<0.05$) content compared to the 1 and 8hr extraction.

Table 1. Comparison of total phenolic content in different solvents extracts of shell and coir in different extraction time periods

Sample	Total phenolic content (mg GAE/g)		
	1 h extraction	8 h extraction	24 h extraction
Shell powder			
Ethanol extract	$46.09^{d, a}$	$47.89^{c, a}$	$40.14^{c, a}$
Methanol extract	$10.19^{e, e}$	$13.08^{e, de}$	$17.05^{e, cd}$
Acetone extract	$15.90^{f, cd}$	$19.95^{d, c}$	$29.19^{d, b}$
Coir powder			
Ethanol extract	$88.07^{c, f}$	$216.06^{b, d}$	$173.86^{b, e}$
Methanol extract	$848.46^{b, c}$	$1012.33^{a, ab}$	$817.43^{a, c}$
Acetone extract	$878.52^{a, bc}$	$1035.61^{a, a}$	$818.4^{a, c}$

Mean with different first letters within the same column and second letters within the same row indicates significant difference ($p<0.05$) according to Duncan's multiple range test.

Shell and coir extracts were found to inhibit the growth of all the bacterial strains tested which was shown as the clear zone on Mueller Hinton's media in a petri dish. Data were presented as mean of three replicates. Mean followed by different letters in the same column indicate significant differences between the different solvents extract of shells and coirs, thus indicate the strength of antibacterial activity among solvents extracts. The diameter of inhibition zones of the different solvent extracts against five pathogenic strains were demonstrated in various sizes, ranging from 7.5 to 11.75 mm and 9.25 to 17.00 mm for the coconut shells and coirs extracts, respectively (Table 2). The inhibition zone indicates the susceptibility of the microbes to the extract. Inhibition zones with a size exceeding 7 mm in diameter indicate that the microbes are susceptible to the extract which means that the extract has antimicrobial activity (Nascimento

et al., 2000). The largest inhibition zone size was recorded by ethanolic coir extract against *S. aureus,* which was 17 mm, followed by methanolic shell extract (11.75 mm) against *E. aerugenes*, acetone coir extract (11.50 mm) against *E. aerugenes* and methanolic shell extract (11.25 mm) against *L. innocua*. While the smallest inhibition zone appeared in ethanolic shell extract against *B. cereus* (7.75 mm) and *C. sakazakii* (7.50 mm). The antibacterial activity of coir and shell extracts was almost equivalent to the control antibiotic, penicillin against *B. cereus* and *E. aerugenes* strains, but slightly lower against *L. innocua* and *S. aureus* strains. However, it was found that *C. sakazakii* strain was resistant to penicillin where no inhibition zone was produced. The antibacterial activity of coconut husk has been reported by Akinyele et al. (2011) against 24 tested bacteria, including *E. coli, S. aureus, S. faecalis, P. aeruginosa, K. pneumoniae, P. vulgaris* and *E. faecalis* with the inhibition zones ranging between 11 to 20 mm in aqueous extracts and 12 to 18 mm in hexane extracts. Meanwhile, according to Rajeev et al. (2011), endocarp or coconut shell extract showed antibacterial activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus* and *M. luteus* with inhibition zone values ranging from 11 to 16 mm.

Table 2. Diameter of inhibition zone of different solvents extracts of coconut shell and coir against five pathogenic strains

Sample	Diameter of inhibition zone (mm)				
	B. cereus	*C. sakazakii*	*E. aerugenes*	*L. innocua*	*S. aureus*
Shell powder					
Ethanol extract	7.75±0.05^{c}	7.50±5.00^{a}	10.00±0.81^{c}	9.25±0.95^{d}	9.75±0.50^{c}
Methanol extract	9.00±1.14^{bc}	9.50±0.57^{a}	11.75±0.50^{ab}	11.25±0.50^{b}	10.25±1.25^{c}
Acetone extract	10.00±0.00^{ab}	9.50±0.57^{a}	10.75±0.95^{bc}	10.50±1.00^{bc}	11.00±1.63^{c}
Coir powder					
Ethanol extract	10.50±1.29^{ab}	9.25±0.50^{a}	10.25±0.95^{bc}	9.50±0.57^{cd}	17.00±3.74^{b}
Methanol extract	9.75±0.50^{ab}	10.25±1.25^{a}	10.25±0.50^{bc}	10.25±0.50^{bcd}	11.00±0.00^{c}
Acetone extract	10.50±0.57^{ab}	9.75±0.50^{a}	11.50±1.29^{abc}	10.25±0.50^{bcd}	11.00±0.81^{c}
Penicillin	11.00±0.00^{a}	0^{b}	12.50±0.70^{a}	13.00±0.00^{a}	43.50±0.70^{a}

Mean followed by different letters within the same column indicates significant difference ($p<0.05$) according to Duncan's multiple range test.

In the antimicrobial study, minimum inhibitory concentration (MIC) refers to the lowest concentration of extract that will inhibit microbial growth, while minimum bactericidal concentration (MBC) indicates the lowest concentration of the extract that can kill tested microbes (Mehta et al., 2013). The MIC and MBC values of coconut coir and shell extracts are shown in Table 3. The MIC and MBC values of the extracts against tested strains ranged between 0.78 to 50 mg/mL for shell extracts and 1.56 to 50 mg/mL for coir extracts. All coir extracts have lower MIC value (6.25 mg/mL) compared to all shell extracts (12.50 mg/mL) against *S. aureus*. But against *E. aerugenes* and *B. cereus*, the MIC value of the methanolic and acetone shell extract were lower (0.78 and 12.5 mg/mL, respectively) than the ethanolic shell extract (3.12 and 50 mg/mL, respectively). Same results were observed in coir extracts where methanolic and acetone extract showed lower MIC against *E. aerugenes*. There was no difference in MIC value among solvent coir extracts against *S. aureus* and *B. cereus*. Meanwhile, for MBC, there was no significant difference between coir and shell extract against *S. aureus* and *B. cereus* (12.5 and 50 mg/mL, respectively), but against *E. aerugenes*, MBC value in methanolic and acetone shell and coir extract were lower (0.78 and 1.56 mg/mL, respectively) compared to ethanolic extract (3.12 mg/mL). MIC values in aqueous and hexane coconut husk extracts as reported by Akinyele et al. (2011) ranged between 0.6 to 5.0 mg/mL and 0.3 to 5.0 mg/mL respectively. Meanwhile Cyriac et al. (2013) reported higher MIC and MBC values in coconut husk aqueous extract ranged between 50 to 75 mg/mL and 75 to 100 mg/mL, respectively.

The diversity of the values in inhibition zones, MIC and MBC implies the strength and differences of phytochemical compounds in a plant extract against a particular bacterial strain (Saad et al., 2014).

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of coconut shell and coir extracts

Sample	MIC (mg/mL)		
	S. aureus	*E. aerugenes*	*B. cereus*
Shell powder			
Ethanol extract	12.50	3.12	50.00
Methanol extract	12.50	0.78	12.50
Acetone extract	12.50	0.78	12.50
Coir powder			
Ethanol extract	6.25	3.12	50.00
Methanol extract	6.25	1.56	50.00
Acetone extract	6.25	1.56	50.00
Shell powder		MBC (mg/mL)	
Ethanol extract	12.50	3.12	>50.00
Methanol extract	12.50	0.78	>50.00
Acetone extract	12.50	0.78	>50.00
Coir powder			
Ethanol extract	12.50	3.12	>50.00
Methanol extract	12.50	1.56	>50.00
Acetone extract	12.50	1.56	>50.00

This antimicrobial activity was contributed by plant phenols and secondary metabolites. Coconut shell and coir contain tannins, saponins, steroids, lignin, and saturated and unsaturated fatty acids (Khalid Thebo et al., 2016; Mazaya et al., 2020), as well as pentosans, cellulose, catechin and epicathecin (Heenataj et al., 2017). Tannin can inhibit microbes by binding to the enzymes and proteins of the cell wall thus leading to the inactivation of the microbe (Rindengan et al., 2006). Meanwhile, both saponin and steroid can cause disruption of lipid membrane and cell wall of the cell (Arabski et al., 2012; Madduluri et al., 2013). Catechin has been reported to possess antibacterial, antiviral, antimutagenic, anti-inflammatory and powerful inhibitor of cellular growth (Heenataj et al., 2017).

Phenolics are abundant in plants with 8,000 structures known so far from simple structures such as phenolic acid to highly polymerised substances such as tannins (Jin and Russel, 2010). Solvent extraction is a commonly used method in obtaining bioactive compounds from plant materials. Solvents consist of polar, semi-polar and non-polar types. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples (Jin and Russel, 2010). High-polar phenolic compounds will be soluble in polar solvents, while less polar compounds will be soluble in non-polar solvents. Several active compounds of different polarity may be present in varying amounts in the extract. Ethanol is more polar compared to methanol while acetone is less polar solvent. Acetone mostly extracted higher molecular weight compounds, while methanol extracted lower molecular weight compounds. The rest of the compounds which are in the middle of molecular weight size mostly

extracted in ethanol solvent. A phytochemical study of coconut fibre from mesocarp part (husk, coir) revealed that ethanolic extract contains phenols, tannins, leucoanthocyanidins, flavonoids, triterpenes, steroids and alkaloids, while butanol extract recovered triterpenes, saponins and condensed tannins. Meanwhile, ethyl acetate extract is rich in polyphenols, catechins, tannins and flavonoids (Esquenazi et al., 2022).

CONCLUSIONS

From the results of the analysis obtained, it can be concluded that the coconut shell and coir have potential antibacterial activities. The usage of different solvents in the extractions affects the phenolic content and antibacterial activity of coconut coir and shell extracts. This study may be used as a guidance in the selection of suitable solvents in the extraction of bioactive compounds from coconut coir and shell. Further detailed research still needs to be done to develop antibacterial or antimicrobial agents from coir and coconut shell.

AUTHORS CONTRIBUTION

MEMN and MFT performed the preparation of raw materials and antimicrobial analysis. NM performed the preparation of raw materials and extractions of samples. MAHS and MFMR carried out the phytochemical analysis.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest.

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