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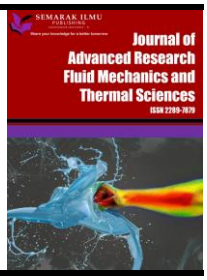
Journal of Advanced Research in Fluid Mechanics and Thermal Sciences

Journal homepage:
https://semarakilmu.com.my/journals/index.php/fluid_mechanics_thermal_sciences/index
ISSN: 2289-7879




# Optimization of Extraction Solvents on the Antioxidant Properties of Coconut Waste

Husna Filzah Ismail[1], Fazrena Nadia Md Akhir[1,*], Nor'azizi Othman[2], Hirofumi Hara[3]

[1] Department of Chemical and Environmental Engineering, Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia
[2] Department of Mechanical Precision Engineering, Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia
[3] Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan

**ARTICLE INFO**

***Article history:***
Received 17 December 2023
Received in revised form 14 April 2024
Accepted 22 April 2024
Available online 15 May 2024

***Keywords:***
Coconut waste; phenolic compound; antioxidant activity

**ABSTRACT**

*Cocos nucifera L.* (family Arecaceae) commonly known as coconut is considered as an important fruit crop in tropical countries and are widely used for therapeutic and domestic purpose. They have effective properties such as antioxidant, antitumor, antiseptic and antimicrobial. The growing demand for green coconut water consumption and food industries cause the dumping of the shell and husk of this fruit, generating large amount of solid waste. This study utilized part of coconut waste to study the impacts of solvent type on the phenolic content. Six different polarities of solvent were chosen. Total phenolic content was performed using Folin-Ciocalteu assay and free radical scavenging activity with 2,2-diphenyl-1-picrilhydrazil (DPPH) method. Total phenolic content for coconut shell was the highest at 71.57 ± 0.275 mg GAE/g for propanol extract and 74.10 ± 0.741 mg GAE/g for acetone extract of coconut husk. The antioxidant activity of all sample extracts was analysed using DPPH assay. Highest radical scavenging activity of coconut husk was observed by propanol extract with 93.82 ± 0.052 % while the lowest scavenging activity was demonstrated by chloroform extract with percentage of 57.77 ± 2.255%. Acetone extract of the shell exhibited the highest scavenging activity of 91.23 ± 0.073%, while chloroform extract of coconut shell demonstrated the lowest antioxidant activity of 70.627 ± 0.467%.

## 1. Introduction

*Cocos nucifera*, commonly known as coconut, is an important fruit crop in tropical countries and is widely cultivated for food industries and green coconut water consumption [1]. They are widely utilized in many parts of the world, particularly in the coastal regions due to their medicinal and nutritional properties. However, despite having an important economic role, 80 to 85% of the fruit weight from the coconut industry such as shell and husk are discarded, generating a huge amount of waste [2]. Coconut shells have similar composition of wood and may take up to 10 years to

* *Corresponding author.*
*E-mail address: fazrena@utm.my*

https://doi.org/10.37934/arfmts.117.1.109117

decompose [3,4]. High residues volume and slow decomposition of these waste may attract diseases vector such as mosquitos, flies, rats, and cockroaches which pose risk to human health. Improper management of such waste will also cause the occurrence of diseases such as dengue and cholera as their accumulation may cause blockage in public drainage, air pollution as well as poor hygiene of public spaces [5,6].

Coconut fruit has unique properties where its wall consists of three layers; exocarp, mesocarp and endocarp [1,7,8]. The mesocarp are hard and fibrous because of the extensive linking between the phenolics, polysaccharides and lignin [7]. Studies demonstrated that different parts of the coconut are used as traditional medicines in different regions of the world for different diseases. For instance, coconut husk was also traditionally used as an herbal chewing stick to clean teeth as an alternative for plastic bristle brushes [9]. Coconut husk also has been widely utilized as a traditional medicine in treating diarrhea and arthritis in Brazil [10]. The shell was used as raw material for activated charcoal and liquid smoke [11].

In recent years, due to their possible uses in food, pharmaceutical and chemical industries, focus has been increased to mainly extract the bioactive compounds from natural sources. These compounds have many effects for human health, such as a reduction in the prevalence of cancer, diabetes, and cardiovascular disease, as well as their anti- mutagenic, anti-inflammatory, and anti-microbial action, which has high potential in industries such as pharmaceutical, cosmetics, and nutraceutical industries [12,13]. Toh *et al.,* [13] reported that extraction conditions such as the solvent type is the contributing factor towards the efficiency in obtaining antioxidant compound from natural resources with highest yields.

Previously, most studies have only focused on either the husk or the coconut shell. Moreover, only a few studies examined the effects of different solvent polarity on the total phenolic recovery of coconut waste and its bioactivity. In addition, most studies only used solvents of similar polarity. The novelty of this recent study aims to fully utilised both part of coconut shell and husk and using solvent of different polarity for the extraction of bioactive compound. This study provides an overview on how coconut waste can be considered as a potential candidate as a potential antioxidant agent to curb the issue of potential side effect of existing synthetic antioxidants. This study will investigate the effects of extraction solvents on the phenolic compound content and the antioxidant activity of coconut waste extract.

## 2. Methodology

### *2.1 Preparation and Extraction of Sample*

The mesocarp (husk) was separated from the endocarp (shell) and washed to remove impurities prior to cutting it into smaller pieces. The collected husk and shell were sun dried and pulverized into a fine powder using grinder (Crusher D3V-10). The powder was kept in an airtight container and kept in a dark and cool place until analysis. Samples were extracted using six solvents of different polarity (methanol, propanol, ethyl acetate, acetone, chloroform, and distilled water). 10 g of coconut shell and husk powder were extracted in 100 ml of each solvent of 70% concentration. The extracts were incubated and agitated at 100 rpm for 48 hours. Then, the extracts were vacuum filtered and kept at 4 $^{o}$C for analysis.

### *2.2 Extraction Yield*

The yield of extraction was calculated as follows:

$$Extraction\ yield\ y(\%) = \frac{Weight\ of\ extract\ after\ evaporating\ solvent}{Dry\ weight\ of\ the\ sample} \times 100\% \quad (1)$$

### *2.3 Total Phenolic Content (TPC)*

The total phenolic content of was determined by Folin-Ciocalteau reagent method with modification. 5 mg/ml stock solution of gallic acid was prepared by dissolving 0.5 gram of dry gallic acid in 10 ml of ethanol and was diluted to 100ml with distilled water. Each 0.25 ml of extract and standard were diluted with 2.25 ml distilled water. 0.25 ml Folin-Ciocalteau reagent was added to each sample and were mixed well and allowed to stand for 5 minutes. 2.5 ml of 7.5% sodium carbonate was added to neutralize the sample. The solution was mixed well and incubated in a dark room for 90 minutes. The absorbance was measured 765 nm using spectrophotometer. The phenolic content was expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry weight of extract.

### *2.4 Antioxidant Assay*

The antioxidant activity of coconut husk and shell were analyzed using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay with slight modification. 0.004% of methanolic DPPH was prepared by dissolving 4mg of DPPH in 100 ml methanol. 2 ml of DPPH solution was then added to 1ml of extract or standard of different concentration (2-20 mg/ml). The solution was well mixed and left in the in the dark for 30 min. The absorbance was read at 517 nm using UV-VIS spectrophotometer (Hach DR6000). Ascorbic acid was taken as standard. The antioxidant activity was calculated using the formula:

$$Radical\ scavenging\ activity\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\% \quad (2)$$

where, $A_{control}$ is the absorbance of the control and $A_{sample}$ is the absorbance of sample extract.

### *2.5 Statistical Analysis*

The analysis was performed in a triplicate manner. Results were expressed as mean standard deviation (SD) for each sample. Analysis of variance (ANOVA) and Dunnett's multiple comparisons test were used to determine significant differences between values ($p<0.0001$) obtained for antioxidant activity, with ascorbic acid as the reference samples. GraphPad Prism 9 version 9.00 for Windows was used for statistical analysis.

## 3. Results

### *3.1 Yield of Extraction*

Visual representation of coconut husk and shell extracted with different polarity of solvents in Figure 1 showed that extract of coconut husk has deeper, and darker colour compared to the shell extract. The extraction yields of coconut husk and shell extract using different polarity of solvents are

shown in Table 1. Propanol extract of coconut shell exhibited the highest extraction yield (25%) followed by acetone extract (19.33%), ethyl acetate (18.00%), methanol (17.33%), chloroform (15%) and lastly distilled water (10%). Meanwhile, for coconut husk, highest extraction yield was shown by methanol (24%) extract while lowest yield was shown by distilled water (12.67%). Similar results were reported by Ismail *et al.,* [14] where highest extract yield of pomegranate peel was obtained from ethanol (24.23%) followed by acetone (21.2%), methanol (20.81%) and water (14.36%).

Higher yield of other solvent compared to distilled water extract for both husk and shell demonstrates that the yield of the extract can be enhanced by adding water in the solvent system. This is due to the greater solubility of sample matrices such as protein and carbohydrates in aqueous solvents system which allow the phenolic compound to be released to the medium [15]. The presence of water increased the bulge in plant material, causing the increase of the contact surface area between the plant matrix and the solvent [16].

**Table 1**
Percentage yield of coconut shell and husk extract

| Solvents | Percentage Yield | |
|---|---|---|
| | Shell | Husk |
| Distilled Water | 10.00 | 12.67 |
| Methanol | 17.33 | 24.00 |
| Propanol | 25.00 | 20.33 |
| Ethyl Acetate | 18.00 | 17.33 |
| Acetone | 19.33 | 20.00 |
| Chloroform | 15.00 | 17.67 |

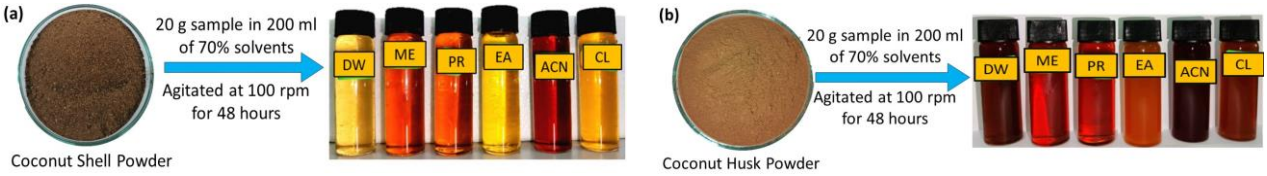



**Fig. 1.** Visual representation of (a) coconut shell extract (b) coconut husk extract in the order of decreasing polarity from left to right (ME: Methanol, PR: Propanol, EA: Ethyl acetate, ACN: Acetone, CL: Chloroform, DW: Distilled Water)

### *3.2 Effect of Solvent on Total Phenolic Content (TPC)*

Phenolic compounds are among the largest classes of bioactive compound with diverse and important biological function [17,18]. They contain 1 or more aromatic rings along with 1 or more hydroxyl groups in their basic structure and contribute to the nutritional quality of fruits or vegetable [17]. They possessed versatile properties including long-term cardiovascular diseases safety, chemoprotective activity, antioxidant, inflammatory potency protection, anticarcinogenic and antimutagenic and their abilities in the prevention and treatment of cancer and various diseases related to oxidative stress [17,19]. They are also capable of replacing synthetic preservatives as they can scavenge free radicals and prevent oxidation in food [19].

Figure 2 showed the TPC recovered from coconut shell and husk extract varied according to the solvent type used. The TPC of the extract ranged from 43.29 ± 0.075 mg GAE/g to 71.59 ± 0.275 mg GAE/g for coconut shell and from 55.24 ± 0.539 mg GAE/g to 74.10 ± 0.741 mg GAE/g for coconut husk. Optimization of solvent type resulted in maximum phenolic content of 71.59 ± 0.275 mg GAE/g for coconut shell extracted with propanol. Meanwhile for the coconut husk, regardless of the highest extraction yield of the methanolic extract (Table 1), its acetone extract resulted in the highest TPC of

74.10 ± 0.584 mg GAE/g (Figure 2) despite its lower polarity compared to methanol. The high efficiency of the acetone extract is attributed to the capability of the acetone-water mixture to degrade the polyphenol-protein complexes, freeing the phenolic compounds [20]. Moreover, by adding water to the organic solvents, a more polar medium is created, facilitating the extraction of chemicals that are soluble in organic solvents [20].

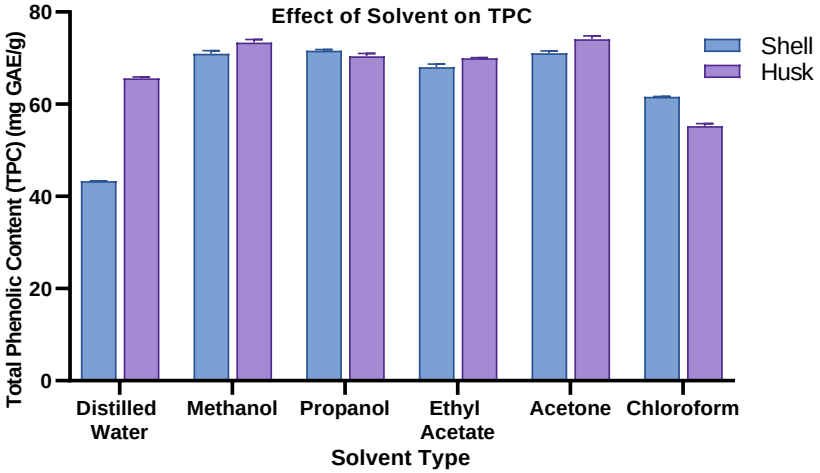



**Fig. 2.** Total phenolic content recovered from coconut husk and shell using different polarity of solvents

Meanwhile the lowest TPC was observed in distilled water extract for shell (43.29 ± 0.075 mg GAE/g) and chloroform extract for husk (55.24 ± 0.539 mg GAE/g). The lower TPC for the distilled water is due to efficacy of pure water as extraction solvent is lowered since phenolic compounds are frequently more soluble in organic solvents less polar than water [21,22]. In addition, this present study also showed that alcoholic extract for both coconut husk and shell have higher TPC compared to its aqueous extract. Similar results were reported by previous studies, where alcoholic extract of pomegranate peel has higher TPC than its aqueous extract, indicating that a 30:70 ratio of water to alcoholic extraction as a viable technique to enhance the extract recovery [14].

The TPC of methanolic extract of coconut husk in this study was 73.38 ± 0.658G mg GAE/g and 70.93±0.681 mg GAE/g for coconut shell which was lower than studies by El-Zawawy [22] with TPC of 113.14 mg GAE/g for the crude methanol extract. The TPC of methanolic extract for coconut husk in this study was higher than its ethyl acetate extract (69.95 ± 0.159 mg GAE/g). These results differed with the studies by Muritala *et al.,* [23] where the TPC for methanolic husk extract was lower (126.7 ± 5.36 mg gallic acid per gram) compared to its ethyl acetate fraction (249.2 ± 17.51 mg gallic acid per gram). Ethanolic and aqueous extract of coconut shell by Tyagi *et al.,* [8] phenolic content of 37.57 mg GAE/g and 23.71 mg GAE/g respectively. The present study also showed a similar result with methanolic extract of coconut shell showing higher TPC compared to the distilled water extract. The difference in findings may be due to the different extraction methods and solvents used [16]. Moreover, the polarity variation of the solvents causes it to selectively extract different hydrophilic or hydrophobic compounds in the sample [24].

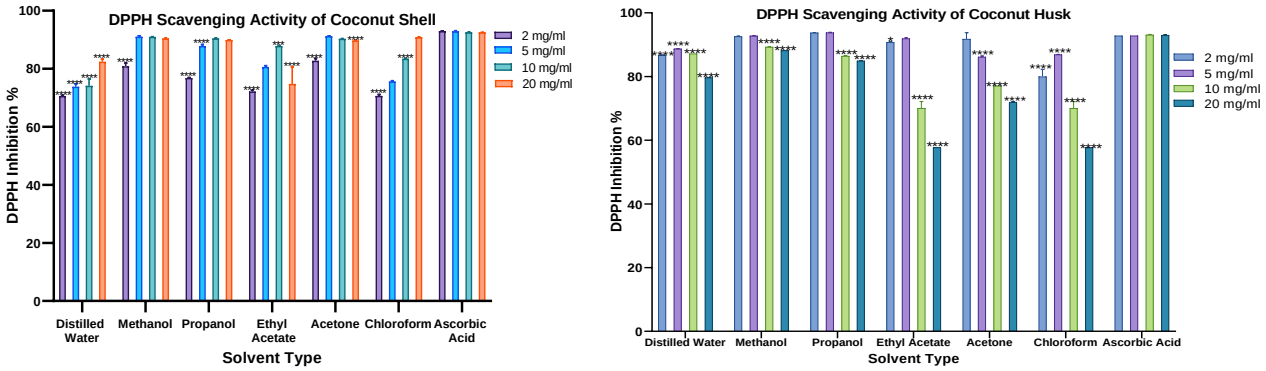
The results also demonstrated that different parts of biomass waste contributed to the variation of phenolic compound content. Comparing the TPC based on the coconut waste part for this study, their husk extract exhibited higher TPC compared to their shell extract using all solvents except propanol and chloroform extract. The differences can be clarified by the differences in the phytochemical composition in the various parts of the plant This is because the phytochemical varies depending on the part of the fruit, where more amount of these compound is likely to be found

accumulating in the fruit epidermis such as the peels, hulls, shell, skin and husk, as a protection of the inner tissues from the sun's UV rays and as defense mechanism against pathogens and predators [21,25]. These results also further proven that the extraction solvents play a significant role for the phenolic compounds extraction from the sample.

### *3.3 Antioxidant Activity of Coconut Waste*

Antioxidant is any chemical substances such as natural body products and nutrient that are capable to neutralize the effect of oxidant of free radicals and other substances [7]. Antioxidants help to slow the oxidation mechanism of oxidizing molecules [26]. In the oxidation, electrons or hydrogen are moved from one species to an oxidizing agent. Free radicals are generated due to oxidation and chain reactions may cause cell damage or even death. Free radicals cause tissue destruction and several pathogenic diseases, including central nervous system degenerative disorders such as Alzheimer's disease, diabetes mellitus, ageing and hypertension [26]. Besides, antioxidants play a part in both food systems and to reduce oxidative stress in the human body. Therefore, since the use of synthetic antioxidants has been suspected to cause harm to human health, antioxidants from natural sources have gained more attention.

Sample extract of four different concentration were analysed to identify the impact of the solvent type and the extract concentration on the antioxidant activity of the coconut waste. The evaluation was conducted by the DPPH method, employing a spectrophotometer, which operates on the principle that the purple colour of DPPH is reduced to light yellow as a result of the donation of hydrogen atoms by the antioxidants, thereby forming stable DPPH radicals [27]. Results in Figure 3 showed that, the radical scavenging activity of coconut shell and husk were concentration dependent. A gradual increase was observed in the DPPH radical scavenging activity of coconut husk and shell with an increase in the extract concentration. However, a decrease in the scavenging activity of coconut husk extract beyond 5 mg/ml was observed for all solvents except acetone extract which showed decrease in inhibition activity beyond 2mg/ml concentration (Figure 3(b)). The highest radical scavenging activity of coconut husk was observed by propanol extract with 93.82 ± 0.052% at concentration of 5 mg/ml compared to the standard ascorbic acid of 92.83 ± 0.016 % scavenging activity of the same concentration. Lowest scavenging activity was demonstrated by chloroform extract with percentage of 57.77 ± 2.255 at 20 mg/ml of extract.




(a) (b)

**Fig. 3.** DPPH Scavenging Activity of (a) Coconut Shell and (b) Coconut Husk
****indicates p<0.0001; *indicates p<0.001 when compared with ascorbic acid as reference

For coconut shell, decrease in the scavenging activity was observed beyond 5mg/ml for methanol and acetone extract. Meanwhile, propanol and ethyl acetate extract of coconut shell showed a drop in the scavenging activity beyond 10 mg/ml concentration of the extract. As the concentration of the sample extract increases from 2 mg/ml to 20mg/ml for distilled water and chloroform extract, a steady increase was observed. Acetone extract of the shell exhibited the highest scavenging activity of 91.23 ± 0.073% at 5mg/ml of extract concentration compared to ascorbic acid with 92.87 ± 0.245 % of scavenging activity. Chloroform extract of coconut shell demonstrated the lowest antioxidant activity of 70.627 ± 0.467% compared to ascorbic acid with 92.819 ± 0.087% at 2 mg/ml concentration.

The decrease in the scavenging activity was due to the extract had reach maximum scavenging activities These results suggested that the antioxidant activity of coconut shell extracts was closely related to their total phenolic content. Coconut waste with greater level of phenolic content portrayed a higher level of antioxidant activity. Similar trend was observed in the previous study by Muritala *et al.,* [23], in which the higher antioxidant activity of their ethyl acetate fraction of coconut husk was strongly related to their higher phenolic content. Methanol-water extract of garlic husk with highest phenolic content also showed similar trend with the lowest $EC_{50,}$ indicating highest antioxidant activity among all extract studied by Kallel *et al.,* [21]. The highest scavenging activity demonstrated by the acetone extract of coconut shell and propanol extract of coconut husk was almost similar to standard ascorbic acid. This indicates that each extract has a great potential as an antioxidant agent.

## 4. Conclusions

This study suggested that the extraction solvents are an important factor in the extraction of phenolic compound. The solvent polarity has a significant impact on the extraction yield and the total phenolic compound. Propanol and methanol extract exhibited highest percentage yield for coconut husk and shell respectively. Propanol and acetone extract demonstrated the highest TPC for coconut shell and husk respectively. Highest antioxidant activity was demonstrated by propanol extract and acetone of coconut husk and shell respectively. Lowest antioxidant activity of both coconut husk and shell was demonstrated by chloroform extract. This study suggested the antioxidant activity was highly associated with the total phenolic content of the extract. This study also suggested that coconut husk and shell have potential as an antioxidant agent.

## Acknowledgement
This research was funded by a grant from Malaysia Japan International Institute of Technology (MJIIT) Incentives.

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