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Isolation and Purification of Potential Major Active Antioxidant Constituent from *Artocarpus heterophyllus* J33 Variety Rind Extract

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ABSTRACT. *Artocarpus heterophyllus* J33 (AhJ33) is a significant fruit cultivar of *A. heterophyllus* in Malaysia and is extensively farmed. For commercial purposes, the pulp is typically extracted from the skin, which is generally discarded. The primary aim of this study was to extract and identify the principal antioxidant component from the rind of AhJ33 fruit waste for the purpose of converting it into a food product with high antioxidant potential. The DDPH assay was chosen as the antioxidant assessment method for crude extract, fractions derived from column chromatography, and isolated and purified compounds. The identification and elucidation of the extracted and purified active antioxidant compound were conducted utilizing spectroscopic methods, including Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Liquid Chromatography-Mass Spectrometry (LC-MS). The spectral data indicated that the primary active antioxidant component was identified as 3,4-dihydroxybenzoic acid, generally referred to as protocatechuic acid. The extracted and purified chemical may serve as reference material (RM), potentially aiding in the manufacture of certified reference material (CRM) for quality assurance in future nutraceutical product development, aligning with the waste-to-wealth concept.

Keywords: Isolation, purification, antioxidant constituent, artocarpus heterophyllus J33, rind

1. INTRODUCTION

Artocarpus heterophyllus Lam, usually referred to as jackfruit, Ceylon Jack tree, or 'Nangka,' is a significant and widely distributed tree in India, Bangladesh (Hossain & Haq, 2006; Prakash et al., 2009), and Southeast Asia, including Malaysia. In addition to its excellent pulp from mature fruits, the immature fruits and seeds of *A. heterophyllus* are frequently utilized as vegetables (Jagtap et al., 2011). In Malaysia, five recognized varieties of *A. heterophyllus*, specifically J33, are predominantly cultivated. The ripe fruit weight of the *A. heterophyllus* J33 variety ranges from 16 to 20 kg.

The rind or peel constitutes the exterior layer of *A. heterophyllus* (Azad et al., 2007). The rind color of jackfruits may reflect their ripeness, which is referred to as the maturity index. Previous research indicates that the rind transitions in colour from green to yellowish, then to yellow and yellow-brown upon ripening, constituting 71% of the fruit's weight (Shamsudin et al., 2009; Ibrahim et al., 2013). The rind has been documented as a source of food components and employed in nutraceutical applications.

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Early work on the identification of antioxidant compounds from Artocarpus was conducted by Ko et al. (1998) in their study of *A. heterophyllus*. Three prenylflavones, namely cycloheterophyllin, artonin A, and artonin B, have potent antioxidant properties with IC50 values of 2.1, 2.4, and 1.8 μM, respectively. All components were extracted from root bark, and the DPPH scavenging test was employed as an antioxidant assessment method. This study aimed to further scientific knowledge of the principal active antioxidant elements of *A. heterophyllus*, particularly the J33 type. The isolated and purified primary active antioxidant component may serve as reference material (RM), thereby aiding in the production of certified reference material (CRM) for quality assurance in future nutraceutical product development, aligning with the waste-to-wealth concept.

2. METHODOLOGY

2.1. General

The UV-Vis was recorded on ELISA Spectramax Plus (Molecular Devices). Fourier Transform Infrared Spectroscopy (FTIR) was recorded on Perkin Elmer Spectrum One FTIR spectrometer (Shelton, USA**)**. The mass value was obtained from mass analyzer 6224 TOF LC/MS Agilent Technologies consisting electrospray (ESI) source system. The ^{1}H NMR and ^{13}C NMR were analyzed on a Bruker 300 Ultrashield NMR Spectrometer measured at 300 and 75 MHz, respectively. The Deuterated acetone was used and chemical shifts (δ and δ_c) were given in ppm. The solvents was evaporated using Buchi Rotavapor R210 at 45ºC. The column chromatography fractions were analyzed using Analytical TLC utilized DC-Plastikfolien $60F_{254}$ (Merck 5735 and 5559). Silica gel 60 (Merck 7734) was used for open column chromatography.

2.2. Pre-extraction

Artocarpus heterophyllus fruit skin J33 (10 kg) were collected from 'Taman Kekal Pengeluaran Makanan' Lancang, Pahang, Malaysia. The rind part was separated from the fruit, cut into small pieces, oven dried at 40 °C for 48 hours and then ground into fine powder.

2.3. Extraction Process

The material was extracted using maceration with 70% aqueous ethanol, as previously described for extraction efficiency (Daud et al., 2018). A 1-kilogram sample of dried rind powder was macerated with 2 L of 70% aqueous ethanol for 72 hours at room temperature (25°C), with periodic agitation. The extract was filtered using Whatman No. 1 filter paper. The marc was re-extracted utilizing the identical process. The solvent was evaporated to obtain the crude extract.

2.4. Determination of Antioxidant Activity

The assessment of antioxidant activity was conducted using radical scavenging activity as outlined by Daud et al. (2018). The antioxidant capacity of the crude extract from AhJ33 fruit rind and the extracted pure components was evaluated based on their scavenging activity against the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. All test samples were produced by dissolving 1.0 mg of materials in 1.0 ml of 70% aqueous ethanol. A DPPH solution was formulated by dissolving 5.0 mg of DPPH in 2.0 ml of methanol, and the solution was stored in the dark at

ambient temperature (25°C). The test specimens were produced in 96-well microtiter plates. Five microliters of methanolic DPPH solution were added to each well. The plate was agitated to guarantee complete amalgamation prior to being positioned in the dark and enveloped in aluminum foil. Following a 30-minute interval, the optical density of the solution was assessed utilizing an ELISA reader, Spectramax Plus (Molecular Devices), at a wavelength of 517 nm. The percentage of inhibition by the sample treatment was assessed by comparing it to the methanol-treated control group. The concentration of the sample necessary for 50% inhibition (IC50) was determined from the radical scavenging graph at various concentrations. All test analyses were conducted in triplicate, and the readings were averaged. Quercetin (Sigma, USA) used as the standard (positive control). The DPPH method quantifies antioxidant activity through percent inhibition.

Percent inhibition = [(Abs control–Abs sample)/Abs control)] 100

where,

*$Abs_{control}$ = absorbance of DPPH radical + methanol

*Abs_{sample} = absorbance of DPPH radical + sample extract/standard (positive control)

2.5. Chromatographic methods

Open column chromatography is performed utilizing glass chromatography columns, which are slurry-packed with normal phase Silica gel 60 (Merck 7734) and eluted via gradient elution. The eluted components were monitored using TLC and observed under UV illumination at 254 and 366 nm for UV-absorbing or quenching compounds and assessed based on their TLC profiles.

2.6. Isolation and purification of most active antioxidant compound

The rind maceration crude extract (RDM) was evaluated for antioxidant potential using radical scavenging assay (DPPH) exhibited value of 94% inhibition as reported earlier in our previous study (Daud et al 2018). From these, 100g of RDM was then resuspended in distilled water and solvent partitioned into dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and water with EtOAc fraction was found to possess the most active activity with value of 94% inhibition.

The EtOAc fraction (50.0g) was first subjected to normal phase open column chromatography (CC) using glass column (2.5cm x 20 cm) silica gel. Solvent elution started with 100% chloroform ($CHCl_3$) and the polarity was slowly increased by adding 20%, 50% and 100% methanol yielding total of 18 fractions. Each fraction (250 ml) was concentrated using a rotary evaporator and subjected to TLC analysis. Based on similar TLC patterns, fractions 1 to 4 (A1) (0.93g), 5 to 16 (A2) (33.0g), 17 to 18 (A3) (9.0g) were recombined and found that fraction A2 possess the highest activity with value of 94% inhibition. Fraction A2 was further subjected to normal phase open column chromatography glass column (1.5 cm x 30.0 cm) over silica gel. The column was eluted gradiently with $CHCl_3$:MeOH as the solvent system (100% $CHCl_3$ to 100% MeOH) The elution begin with 90% followed by 80%, 50% chloroform and finally 100% methanol. From these, 42 fractions (150ml each) were collected. Each fraction was further concentrated and subjected to TLC analysis. Based on the TLC evaluation, fractions 1-10 (A2-1), 11-27 (A2-2), 28-39 (A2-3) and 40-42 (A2-4) were recombined and A2-1 was found to possess the highest activity with

value of 92%. Fraction A2-1 was subjected to normal phase column chromatography (30.0 cm x 1.5 cm) packed with Silica gel 60 (Merck 7734). The column was eluted gradiently with $CHCl_3$: EtOAc from 50% chloroform to 100% EtOAc. This afforded 450mg of transparent needles of compound (Ah1) as a major compound from active ethyl acetate fraction of A2-1. The structure of the isolated and purified compound was further elucidated using spectroscopic methods together with its antioxidant activity.

2.7. Identification of active compounds

The elucidation and identification of the isolated chemical (Ah1) from the active ethyl acetate fraction were conducted using Fourier Transform Infrared Spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry (LCMS). All ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) investigations were conducted on a Bruker spectrometer equipped with pulsed field gradients (PFG) and an Indirect Detection Probe. Deuterated acetone was utilized, and the chemical shifts (δ and δC) were reported in ppm. The mass spectrum was obtained using a 6224 TOF LC/MS from Agilent Technologies, with an electrospray ionization (ESI) source in negative mode. The intricate structure was depicted with ChemSketch software.

3. RESULTS AND DISCUSSION

3.1. Isolation and purification of major active antioxidant compound

The isolation and purification process of the ethyl acetate sub-fraction (A2-1) produced 450 mg of transparent crystalline needles of compound Ah1. Figure 1 displays the mass spectrum of compound Ah1. Two prominent peaks at m/z 109 and 153 were observed. The molecular ion signal at m/z 153 indicates the chemical formula $C_7H_6O_4$ (Table 1). The chemical formula suggests an index of hydrogen deficit (IHD) of 5, suggesting the potential inclusion of a benzene ring alongside an additional double bond. The fragment ion peak at m/z 109 may result from the elimination of a carboxyl group.

Figure 2 illustrates that the IR spectra of Ah1 exhibited absorption patterns at wavenumbers of 3199 cm^{-1} (broad, OH), 1676 cm^{-1} (carboxylate, C=O), 1529 cm^{-1} (C=C), and 1300 cm^{-1} (C-O). Compound Ah1 may be a phenolic compound containing a carboxyl group, as indicated by the absorptions. Thus, two oxygen atoms are part of the carboxyl group, while two additional oxygens may be associated with the hydroxyl group connected to the benzene ring.

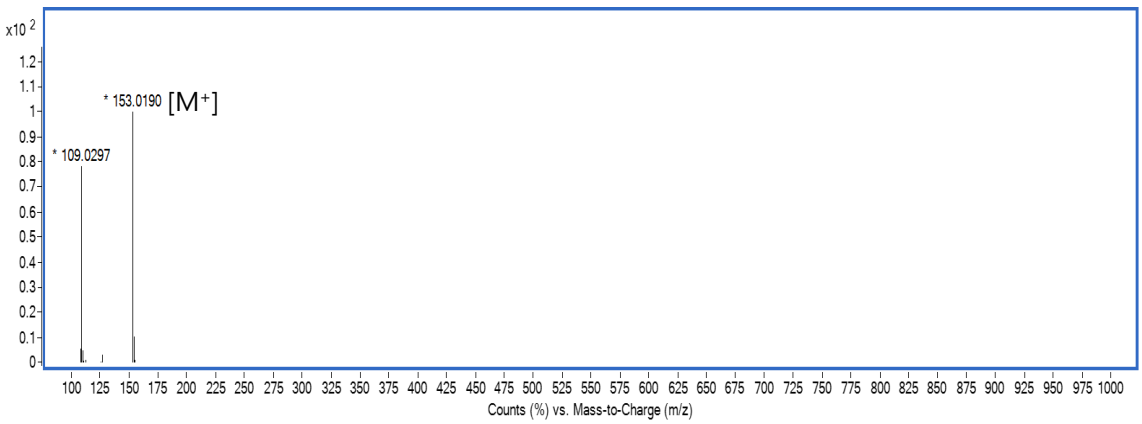


Figure 1. Mass spectrum of compound Ah1.

Table 1. Mass analysis of compound Ah1.

Formula (M)	*m/z* (Observed)	*m/z* (Calculated)	Diff (ppm)	Score (%)
$C_6 H_6 O_2$	109.0297	109.0295	1.79	99.73
$C_7 H_6 O_4$	153.0190	153.0193	2.16	99.36

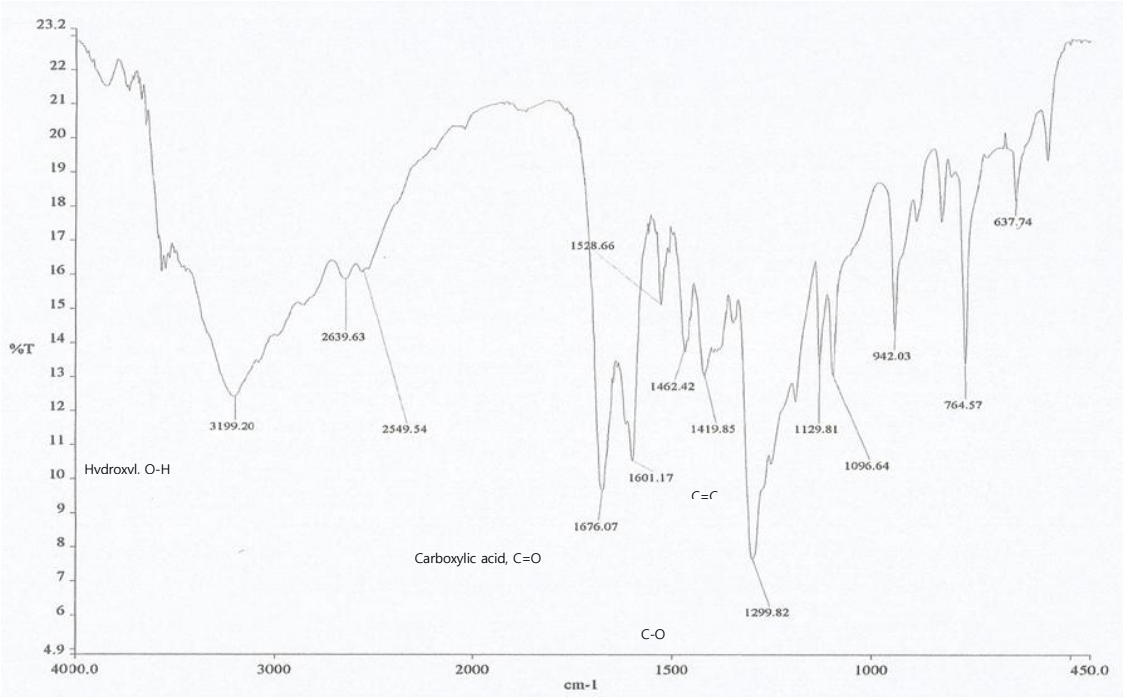
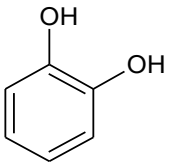
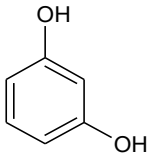


Figure 2. FTIR spectrum of Ah1 (KBr disc).

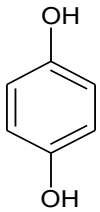
The mass data analysis presented in Table 2 indicates that the m/z value of 109 may correspond to the chemical formula $C_6H_6O_2$, which exists as benzenediol. Theoretically, it may exist as 1,2-benzenediol, 1,3-benzenediol, and 1,4-benzenediol, referred to as catechol, resorcinol, and hydroquinone, respectively, with the structures illustrated below:



1,2- benzenediol



1,3-benzenediol



1,4-benzenediol

The location of the hydroxyl group can be determined using 1H NMR spectroscopy. Figure 3 illustrates the 1H NMR spectrum of Ah1 acquired in deuterated acetone. Three proton signals in the range of δ6.5-8.0 ppm were distinctly seen, indicating the presence of aromatic chemicals (Blunder et al., 2017). A set of methine aromatic protons

exhibiting resonances at δ6.91 (1H, *d, J*= 8.4 Hz), 7.47 (1H, *dd, J*=8.1, 2.1 Hz), and 7.53 (1H, *d, J*=2.1) signifies ortho-coupled, ortho and meta-coupled, and meta-coupled protons, respectively. The protons are part of an ABX spin system. Consequently, the protons at 6.91, 7.47, and 7.53 are provisionally designated as H5, H6, and H2, respectively, in the suggested sub-structure illustrated below. Nevertheless, the carboxylic proton was not detectable. This may result from deuterium exchange of the solvent and dynamic effects on molecular relaxation, as documented by Claridge (2016) and Atta-ur-Rahman et al. (2015).

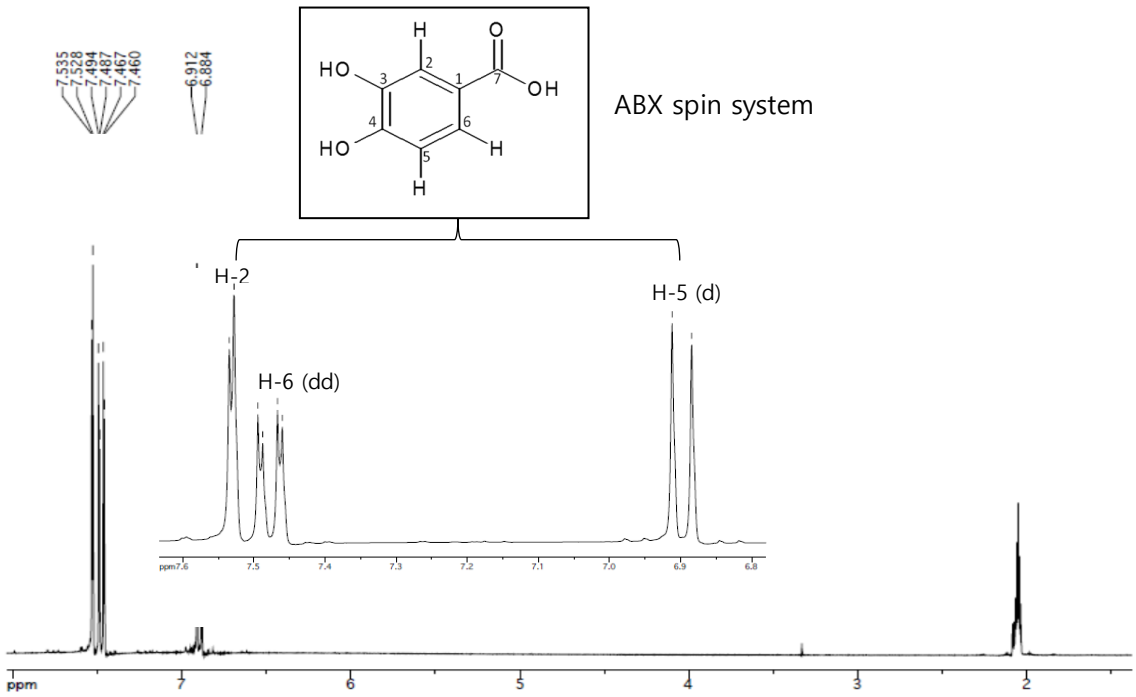


Figure 3. [1]H NMR spectrum of compound Ah1.

Figure 4 illustrates the 13C NMR spectrum of compound Ah1, exhibiting seven peaks within the δc range of 115 to 170 ppm, signifying the existence of aromatic carbons (Pavia et al., 2015). The chemical shift value for non-oxygenated aromatic carbons ranges from δc 100 to 119, but for oxygenated carbons, it spans around δc 120 to 210 ppm. Of the seven peaks, six correspond to aromatic carbons, with two of these carbons observed at δc 150.7 (C-4) and δc 145.6 (C-3) being oxygenated. Thus, the carbon values at δc 115.6, δc 117.4, and δc 123.5 ppm correspond to non-oxygenated carbons at C-5, C-2, and C-6, respectively, whereas the peak at δc 123.1 ppm denotes the quaternary carbon (C-1). The seventh signal detected in the lowest field (δc 167.6 ppm) signifies a carboxylic carbon C-7.

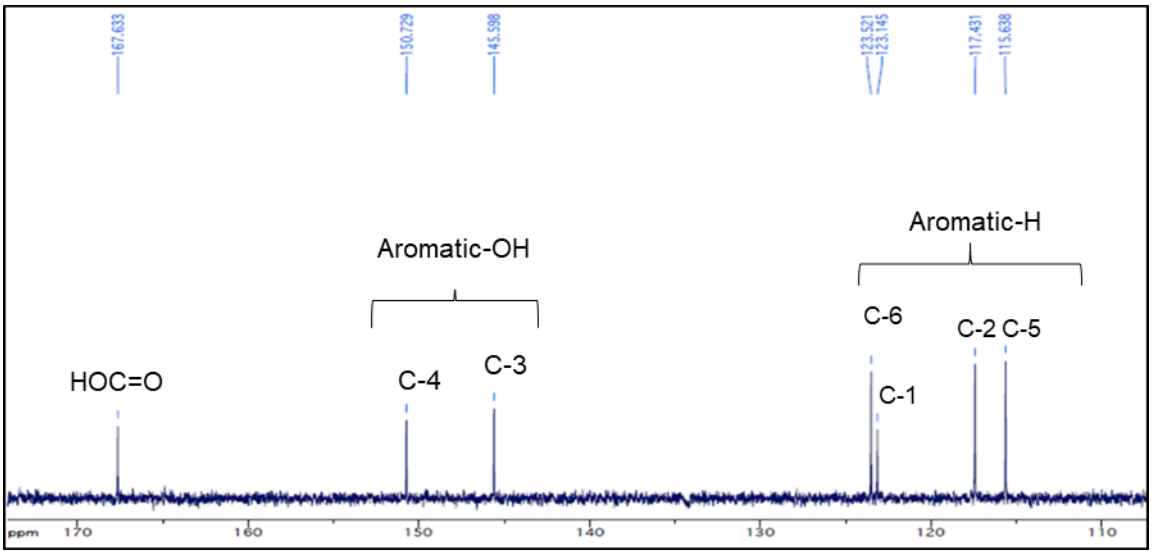


Figure 4. ^{13}C NMR spectrum for compound Ah1.

Table 2 presents a comparative analysis of the Ah1 compound's spectral data from ^{1}H NMR and ^{13}C NMR against existing literature. According to mass, ^{1}H NMR, and ^{13}C NMR data, together with a comparison to existing literature (Da Silva et al., 2015), Ah1 is identified as 3,4-dihydroxybenzoic acid, also known as protocatechuic acid ($C_7H_6O_4$). The structure is illustrated below:

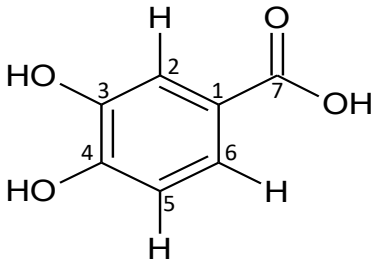


Table 2. ^{1}H and ^{13}C NMR spectral data of compound Ah1 with literature.

Position	Compound Ah1		Literature*	
	δ_H (multiplicity, *J* in Hz)	δ_C	δ_H (multiplicity, *J* in Hz)	δ_C
1	-	123.1	-	123.2
2	7.53 (1H, *d*, *J*=2.1, H-2)	117.4	7.53 (1H, *d*, *J*=2.0, H-2)	117.6
3	-	145.6	-	145.5
4	-	150.7	-	150.6
5	6.91 (1H, *d*, *J*= 8.4, 2.1, H-5)	115.6	6.90 (1H, *d*, *J*=8.3, H-5)	115.8
6	7.47 (1H, *dd*, *J*=8.1, 2.1, H-6)	123.5	7.48 (1H, *dd*, *J*=8.3, 2.1, H-6)	123.7
7	-	167.6	-	167.7

*Da Silva et al. (2015) [Measured in acetone-d 600MHz (^{1}H) and 150 MHz (^{13}C)]

3.2. DPPH radical scavenging activity of Ah1 (protocatechuic acid)

To ascertain if Ah1 was accountable for the antioxidant activity of sub-fraction A2 and fraction A2-1, its antioxidant activity was then assessed employing the DPPH radical scavenging assay. Ah1 had significant radical scavenging activity of 90.2% at a concentration of 1000 µg/ml, as indicated in Table 3. This corresponds to the antioxidant activity values of A2 and A2-1 at identical concentrations. The significant antioxidant activity of the AhJ33 variety rind extract may be ascribed to the presence of protocatechuic acid, which has been identified as the principal compound by a bioassay-guided methodology. Theoretically, the structure of protocatechuic acid, which contains two hydroxyl (OH) groups in the aromatic ring, may enhance antioxidant activity through its capacity to donate hydrogen and disrupt free radical structures (Milenkovic et al., 2017). This strongly suggests that the identification of Ah1 is the primary antioxidant component that significantly contributes to the antioxidant activity of fraction A2 and sub-fraction A2-1 for the J33 variety. Figure 5 indicates that the IC_{50} value was established at 57 µg/ml.

Table 3. DPPH radical scavenging of compound Ah1 at different concentration.

Concentration (ug/ml)	Inhibition (%)
1000	90.2±0.17
500	81.3±0.11
250	70.4±0.12
125	62.5±0.17
60	51.1±0.23
50	47.4±0.22
40	43.3±0.16
30	38.2±0.22

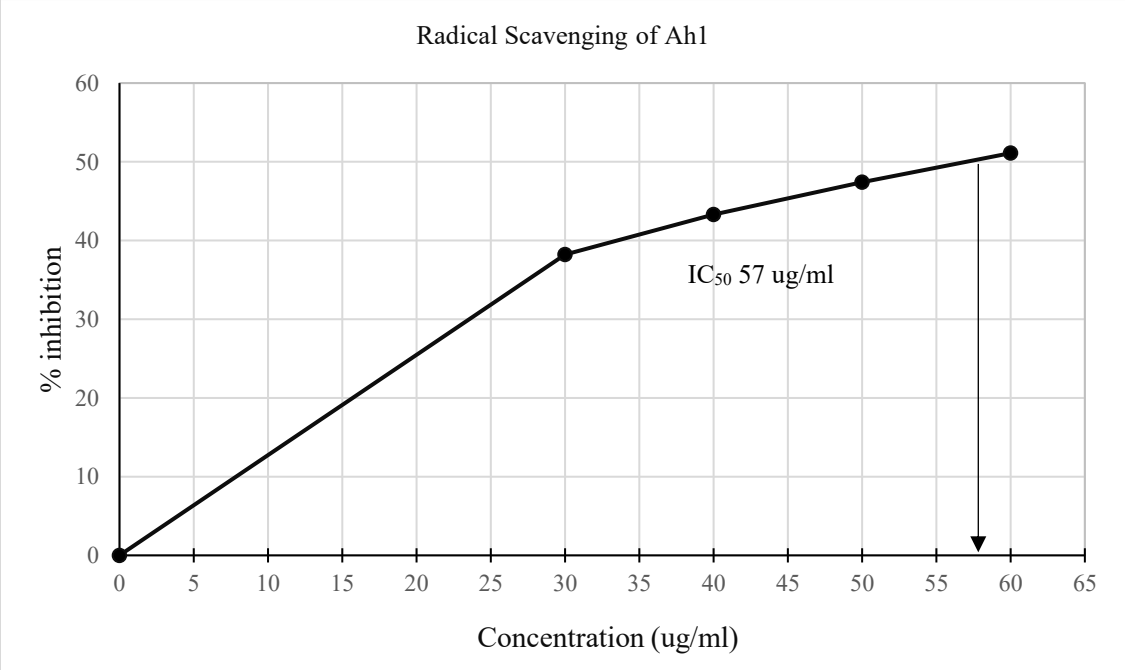


Figure 5. The IC_{50} value of DPPH radical scavenging for compound Ah1.

4. CONCLUSION

The isolation and purification of the principal active antioxidant component from the rind extract of the Artocarpus heterophyllus J33 variety, utilizing chromatographic techniques and guided by the DPPH radical scavenging assay, resulted in the identification of a major compound, 3,4-dihydroxybenzoic acid, commonly referred to as protocatechuic acid, based on MS, IR, 1H NMR, and 13C NMR spectral data. The primary isolated chemical exhibited a robust radical scavenging activity of 90.2% at a concentration of 1000 µg/ml, with an IC50 value of 57 µg/ml. The detected elements may serve as indicators for quality control. This will guarantee the acquisition of only superior-quality extracts in each manufacture.

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AUTHOR CONTRIBUTIONS

Mohd Nazrul Hisham Bin Daud responsible for data collection, LCMS, FTIR and antioxidant analysis, and preparation of the original draft.

Agustono Wibowo is responsible for the NMR analysis.

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DATA AVAILABILITY

Not applicable.

COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

SUPPLEMENTARY MATERIAL

Not applicable.

REFERENCES

Atta-ur-Rahman, Choudhary, M., & Atia-tul-Wahab (2015). Solving Problems with NMR Spectroscopy, Second Edition. Cambridge: Academic Press.

Azad, A.K., Jones, J.G., & Haq, N. (2007). Assessing morphological and isozyme variation of jackfruit (*Artocarpus heterophyllus Lam.*) in Bangladesh. *Agroforest Systems, 71*, 109–125.

Blunder, M., Orthaber, A., Bauer, R., Bucar, F., & Kunert, O. (2017). Efficient identification of flavones, flavanones and their glycosides in routine analysis via off-line combination of sensitive NMR and HPLC experiments. *Food Chemistry, 218*, 600–609.

Claridge, T.D.W. (2016). High-Resolution NMR techniques in organic chemistry 3rd edition. Elsevier Science.

Da Silva, L.A.L., Faquetia, L.G., Reginatto, F.H., Dos Santos, A.D.C., Barison, A., & Biavatti, M.W. (2015). Phytochemical analysis of *Vernonanthura tweedieana* and a validated UPLC-PDA method for the quantification of eriodictyol. *Revista Brasileira de Farmacognosia, 25*, 375–381.

Daud, M.N.H., Wibowo, A., Abdullah, N., & Ahmad, R. (2018). Bioassay-guided fractionation of Artocarpus heterophyllus L. J33 variety fruit waste extract and identification of its antioxidant constituents by TOF-LCMS. *Food Chemistry*, *266*, 200–214.

Hossain, A., & Haq, N. (2006). Jackfruit: *Artocarpus heterophyllu*s. In: Williams, J. T., Smith, R.W., and Dunsiger, Z., Eds. Tropical fruit trees. Southampton UK: Southampton Centre for Underutilised Crops, University of Southampton, 127-139.

Ibrahim, M., Islam, M.S., Helali, M.O.H., Alam, A.K.M.S., & Shafique, M.Z. (2013). Morphological fruit characters and nutritional food value of different jackfruit (*Artocarpus heterophyllus* Lam.) cultivars in Rajshahi region of Bangladesh. *Bangladesh Journal of Scientific and Industrial Research, 48*(4), 287-292.

Jagtap, U.B., Waghmare, S.R., Lokhande, V.H., Suprasanna, P., & Bapat, V.A. (2011). Preparation and evaluation of antioxidant capacity of Jackfruit (*Artocarpus heterophyllus* Lam.) wine and its protective role against radiation induced DNA damage. *Industrial Crops and Products, 34*, 1595–1601.

Ko, F.N., Cheng, Z.J., Lin, C.N., & Teng, C.M. (1998). Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Radical Biology and Medicine, 25*(2), 160–168.

Milenkovic, D., Yorovic, J., Jeremic, S., Markovic, J.M.D., Avdovic, E.H., & Markovic, Z. (2017). Free radical scavenging potency of dihydroxybenzoic acids. *Journal of Chemistry*, *2017*, 1-9.

Pavia, D.L., Lampman, G.M., Kriz, G.S., & Vyvyan, J.R. (2015). Introduction to spectroscopy, fifth edition. Boston, Cengage Learning Press.

Prakash, O., Kumar, R., Mishra, A., & Gupta, R. (2009). *Artocarpus heterophyllus* (Jackfruit): An overview. *Pharmaccognosy Review, 3*, 353−358.

Shamsudin, R., Ling, C.S., Ling, C.N., Muda, N., & Hassan, O. (2009). Chemical compositions of the jackfruit Juice (*Artocarpus*) cultivar J33 during storage. *Journal of Applied Sciences, 9*, 3202-3204.