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Antioxidant Activity of Aceh Curry Leaves (Murraya Koenigii) Extracted Using Various Solvents

Desy Anggriani[1], Lisa Azila[1], Cut Meurah Rosnelly[1], Hisbullah[1], Syaubari[1], Teuku Mukhriza[1,*]

[1] Department of Chemical Engineering, Universitas Syiah Kuala, Banda Aceh, Indonesia

[*] *Corresponding author: mukhriza.tm@usk.ac.id*

Abstract: Free radicals are molecules that are highly reactive and contain unpaired electrons. These molecules are harmful to healthy cells. Antioxidants have the ability to neutralise free radicals by donating one of their electrons to the molecules that make up free radicals. This prevents free radicals from wreaking havoc on healthy cells. Curry leaf, also known as Murraya Koenigii, is a member of the Rutaceae family and is a spice that is commonly used. By gauging the degree to which various solvents and ratios are able to extract antioxidants from curry leaves, the objective of this study is to find the optimal solvent and combination for doing so. A Soxhlet and a solvent mixture consisting of hexane, chloroform, and ethanol in a ratio of 1:10 were used to extract the anti-oxidant components of curry leaves. With a yield percentage of 30.53 percent, the ethanol solvent proved to be the most efficient of the three in terms of extracting antioxidants from the sample. Not only did extracts of curry leaves obtained from the ethanol solvent have the best yield (when compared to extracts derived from other solvents), but they also have the highest levels of inhibition (54.42%) and antioxidant activity (40.667 ppm) when it comes to catching free radicals.

Keywords: *Free radical, antioxidant, extraction, percent inhibition, antioxidant activity*

1. Introduction

Free radicals are the outcome or byproduct of a variety of pollutants, including: cigarette smoke, automotive exhaust, and ultraviolet (UV) radiation. Long-term exposure to free radicals can harm the human body's cells and lead to chronical diseases such as cancer, cataracts, heart attacks, and impaired renal function [1]. These negative impacts of free radical can be prevented by antioxidant compounds that are able to protect body cells from damage [2].

Antioxidants can be found in both natural and synthetic forms. The most commonly used synthetic antioxidants are Propyl Gallate (PG), Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT) and Tertbutyl Hydroquinone (TBHQ). Synthetic antioxidants are carcinogenic that may have adverse health consequences. Studies on BHA and BHT have shown that these components can cause tumors in experimental animals on long-term use [3]. Therefore, natural antioxidant is more favourable since they are able to protect the body against damage caused by reactive oxygen compounds, inhibit the occurrence of disease, and are able to inhibit lipid peroxidation in food.

Many plants have been identified as source of antioxidant including curry leaves (*Murraya koenigii*). *M. koenigii* contains phenolic, flavonoids, and alkaloids which are antioxidant compounds [4]. The antioxidant compounds were extracted from the plant by using various types of solvents. Effectiveness of extraction depends on the solubility of the compound in the solvent. Verdiana et al. [5] reported that ethanol 70% extracted highest antioxidant compounds (more than 50%) from lemon peel compared to aceton 70% and methanol 70%. Other researcher claimed that methanol is the best solvent to extract noni fruits (Morinda *citrifolia* L.)[6]. These results indicate that solvent type influence the quality of extract. Therefore, in this study we examined several types of solvent to extract antioxidant from *M. koenigii.*

2. Materials and Methods

Materials

Curry leaves were obtained from a local market in Banda Aceh. Chloroform 99.8%, ethanol 99.8%, methanol 99.8%, hexane 99.88%, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich.

Method

Sample preparation

Fresh curry leaves were removed from stems and leaf stems. The leaves went through a screening procedure until they were an average of 24 mesh in diameter. A soxhlet device was used to introduce 30 grams of curry leaf powder that had been wrapped in filter paper. The solvents utilized were chloroform, hexane, and ethanol at mass ratios of 1:10 w/v to curry leaves. The soxhletation procedure required up to six hours with chloroform solvent at 61.2 °C, hexane solvent at 68 °C, and ethanol solvent at 78.3 °C. To obtain pure curry leaf extract, the evaporation method was used. The yield percentage was calculate using Eq. (1)

$$\%Yield = \frac{Extract\ weight}{initial\ sample\ weight} x\ 100\% \quad (1)$$

Antioxidant test and FTIR

The DPPH solution was prepared by mixing 8 mg of DPPH powder with 100 ml of methanol. The solution was then covered with aluminum foil and stored in a dark room. Using a UV-Vis spectrophotometer (Shimazu) an absorbance value is determined by combining 3 mL of DPPH solution with 3 mL of methanol. 8 mg curry leaf extract is dissolved in 100 ml of methanol. The solution was then diluted to 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. Then, in a test tube, 3 ml of each solution concentration was added to 3 ml of DPPH solution and the mixture was homogenized. The samples were kept in the dark for one hour. The absorbance value was then determined by measuring the wavelength at which it was most effective using a UV-Vis Spectrophotometer. Using the following formula, the percentage of antioxidants' ability to capture free radicals was determined

$$\%\ inhibition = \frac{initial\ absorbance - sample\ absorbance}{initial\ absorbance}\ x\ 100\% \quad (2)$$

The IC50 value is the amount of antioxidant activity required to eliminate 50% of DPPH radicals. The IC50 value is determined by linear regression of the concentration curve of the sample to the absorbance value, where y = ax + b, with y = 50 and x is IC50 value. FTIR analysis was also performed to observe functional group of the compound contained in curry leaves.

3. Results and Discussion

The effect of solvent type to yield

The percentage of yield obtained from ethanol, chloroform and hexane is listed in Table 1. The results show that ethanol solvent produced the highest antioxidant yield, at 30.53%, when compared to chloroform and hexane solvents. Higher yields are achieved with ethanol than with hexane or chloroform because of ethanol's high polarity as a solvent. In addition, the hydroxyl group of phenolic compounds in ethanol solvent can bond to the hydrogen group of ethanol's hydroxyl group [7].

Table 1. Yield of Curry Leaf Extract with Various Solvent

Solvent	Yield (%)
Ethanol	30.53
Chloroform	23.4
Hexane	14.7

The antioxidant activity of avocado seed extract in ethanol solvent was tested in a study and found a yield percentage of 6.248% for dried avocado seeds [8]. Another research conducted using methanol as

the solvent to extract mangosteen found that the percentage of antioxidant yield was 21% in dry conditions [9]. When compared to other plants, curry leaves extract in this research had 30.53% antioxidants when extracted with ethanol.

Inhibition activity of various solvents

A measure of an antioxidant's effectiveness in preventing free radical damage can be calculated using percent inhibition [10]. This value is determined by analyzing the ethanol, chloroform, and hexane extract of the antioxidant. Table-2 shows absorbance value of the solvents with different concentration.

Table 2. Absorbance value of the solvents with different concentration.

Concentration	Absorbance Value		
(ppm)	Hexane	Chloroform	Ethanol
20	0,316	0.2717	0.2144
30	0.3015	0.2577	0.191
40	0.2612	0.2347	0.1827
50	0.2596	0.2335	0.1749
60	0.2301	0.207	0.1694

Using the information in the table that was just presented, the percentage of inhibition was shown in the graph below using Eq. 2.

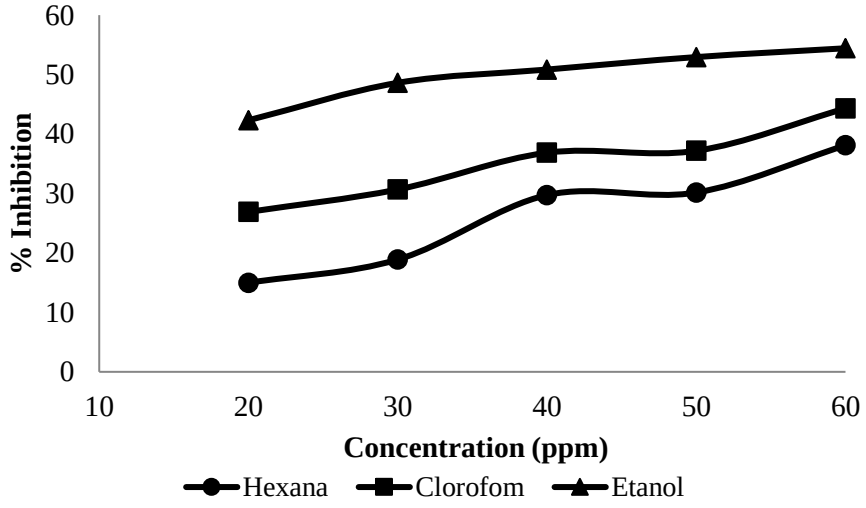

Figure 1. The effect of concentration of various solvent on % inhibition

Figure 1 depicts the percent inhibition of curry leaf extract by hexane, chloroform, and ethanol. According to the diagram above, the fraction of inhibition increases as the concentration of curry leaf extract rises. This indicates that solvents containing varied quantities of antioxidant extracts can give H atoms to decrease free radicals [11]. In chloroform solvent containing 20–60 ppm of antioxidants, the percentage of inhibition ranged from 26.7 to 44.31 %. In hexane solvent with the same quantity of antioxidants, the inhibition percentage ranged from 14.98 to 38.09 percent. In ethanol solvent containing the same antioxidant content, the inhibition percentages were 42.31 and 54.44 percent, respectively. This suggests that the ethanol solvent has a higher proportion of inhibition than the chloroform and hexane solvents.

Antioxidant activity (IC50)

Table 3 provides a listing of the classifications that are utilised to categorise the level of antioxidant activity based on the IC50 value.

Table 3 Category of Antioxidant Strength based on IC50 Value [12]

IC50 Value (ppm)	Antioxidant Properties
< 50	Very Strong
50 – 100	Strong
100 – 150	Intermediate
150 – 200	Weak

It can be seen that IC50 under 50 ppm has a very strong antioxidant property, 50-100 ppm indicate intermediate antioxidant power and 150-200 ppm is the weakest value. Antioxidant activity was calculated using % inhibition graph and the result is illustrated in following table.

Table 4 IC50 Value of curry leave extracted using various solvents

Solvent	IC50 Value
Ethanol	40.67
Chloroform	75.91
Hexane	81.23

According to Table 4, ethanol has higher antioxidant activity than chloroform and hexane. The IC50 value of 40.67, the lowest reported, indicates that this compound has strongest antioxidant effects (50). Chloroform and hexane, meanwhile, are classified only as powerful solvents. The antioxidant content that can be extracted by the three solvents explains this. Antioxidants of the phenolic and tannin kinds can be extracted using ethanol as a solvent. Saponins and steroids were found to be the extracted solvents from chloroform and hexane. Antioxidant activity can be changed depending on the type and amount of antioxidants extracted (IC50). The presence of hydroxyl groups, as well as the number and shape of these groups, are crucial to a molecule's antioxidant action. The contribution of hydrogen atoms is also necessary for antioxidant action [2], [11]. Because of their structure as phenolic compounds, in which compounds with OH groups are connected to the carbon of the aromatic ring, phenolic and tannin-type antioxidants are the most effective antioxidants.

FTIR analysis

The existence of specific functional groups in a substance or molecule can be reliably verified using FTIR analysis. The infrared spectrum region or infrared chart is used to interpret the presence of these functional groupings. Curry leaf extract FT-IR spectra have a pattern that is nearly identical to those of curry powder. This suggests that the constituent chemical components are very similar. The following diagram displays the outcomes of Fourier transform infrared spectroscopy (FTIR) study performed on samples of curry leaf extract in chloroform, ethanol, and hexane.

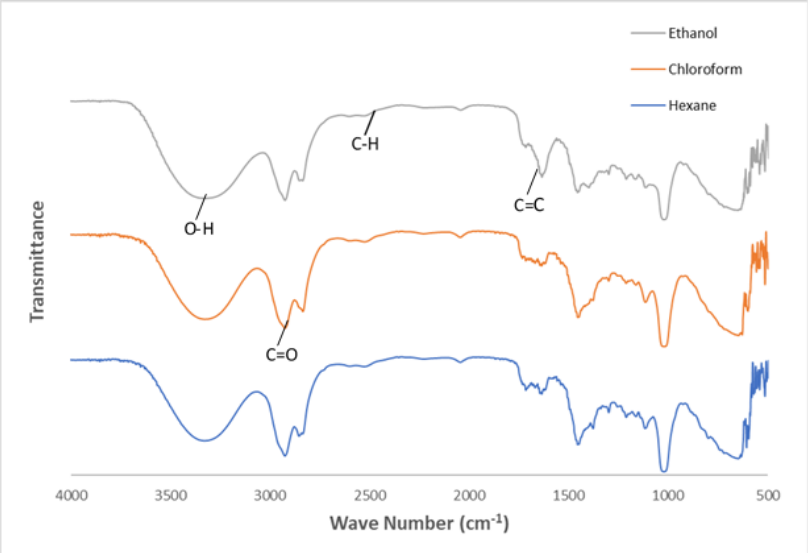

Figure 2. FTIR analysis of curry leaf extract using various solvent

Figure 2 shows that none of the three organic solvents (ethanol, chloroform, and hexane) were present in the extracted curry leaves. In a 1:10 mixture, the functional groups in the three solvents are

almost identical. At wave number 3325.29 cm^{-1}, the O-H functional group was discovered that; at wave number 2922.16 cm^{-1}, the C-H functional group was located; at wave number 2835.36 cm^{-1}, the aldehyde functional group was found; and at wave number 2596.19 cm^{-1}, the carboxylic acid functional group were discovered. There is also a C=C bond (alkene) at 1637.56 cm^{-1}. The following table [13] displays the wave numbers for each functional category.

Table 5. Infrared Wavelengths of Some Functional Groups

Bond	Functional Group	Wavelength cm^{-1}
OH	alcohol	3200-3600
	-H	3210-3550
	Acid	2500-2700
NH	Amine	3300-3700
CH	Alkane	2750-2960
	Alkene	3010-3095
C=C	Alkyne	2140-2260
C=C	Alkena	1620-2680
C=O	Aldehyde	2780-2900
	Ketone	1675-1725
	Ester	1720-2750
C=N	Nitrile	2000-2300
NO	Nitro	1500-1650

According to the functional group analysis, the ethanol, chloroform, and hexane solvents all have phenolic and saponin functional groups, and the curry leaf extract with the three solvents is expected to have the same functional groups as the three solvents. This study discovered that the O-H bond vibrations are absorbed at wave number 3549.02 cm^{-1}. The alcohol group, which is a component of the phenolic molecule, vibrates in the bond. Additionally, using ethanol as the solvent and the FTIR test, Maulana [14] investigated the antioxidant activity of flavonoid components from white guava leaf extract. These findings showed that the O-H functional group in guava leaf extract in ethanol solvent has a wave number of 3350.67 cm^{-1}. Based on this research, it is clear that ethanol solvent is the ideal solvent for extracting antioxidants since it tends to remove the flavonoid compound's O-H functional group. Similar to the preceding explanation, flavonoid molecules have a powerful ability to bind to free radicals.

4. Conclusion

The highest yield of curry leaf extract (30.53%) was obtained using ethanol solvent, and its value of antioxidant activity was 40.67 ppm, out of the three solvents employed. FTIR analyses have determined that the O-H functional group in curry extract is the most powerful flavonoid component in neutralizing free radicals.

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References

[1] Fakriah, E. Kurniasih, Adriana, and Rusydi, "Sosialisasi Bahaya Radikal Bebas dan Fungsi Antioksidan Alami Bagi Kesehatan," *Jurnal Vokasi*, vol. 3, no. 1, p. 1, Jun. 2019, doi: 10.30811/vokasi.v3i1.960.

[2] V. Lobo, A. Patil, A. Phatak, and N. Chandra, "Free radicals, antioxidants and functional foods: Impact on human health," *Pharmacogn Rev*, vol. 4, no. 8, p. 118, 2010, doi: 10.4103/0973-7847.70902.

[3] J. Flieger, W. Flieger, J. Baj, and R. Maciejewski, "Materials Antioxidants: Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles," 2021, doi: 10.3390/ma14154135.

[4] M. Zahin, F. Aqil, F. M. Husain, and I. Ahmad, "Antioxidant Capacity and Antimutagenic Potential of Murraya koenigii," *Biomed Res Int*, vol. 2013, pp. 1–10, 2013, doi: 10.1155/2013/263509.

[5] M. Verdiana, I. W. R. Widarta, and I. D. G. M. Permana, "Pengaruh Jenis Pelarut Pada Ekstraksi Menggunakan Gelombang Ultrasonik Terhadap Aktivitas Antioksidan Ekstrak Kulit Buah Lemon (Citrus limon (Linn.) Burm F.)," *Jurnal Ilmu dan Teknologi Pangan (ITEPA)*, vol. 7, no. 4, p. 213, Dec. 2018, doi: 10.24843/itepa.2018.v07.i04.p08.

[6] D. Ramdani, M. Majuki, and S. Chuzaemi, "Pengaruh Perbedaan Jenis Pelarut Dalam Proses Ekstraksi Buah Mengkudu (Morinda citrifolia L.) Pada Pakan Terhadap Viabilitas Protozoa dan Produksi Gas In-Vitro," *Jurnal Ilmu-Ilmu Peternakan (Indonesian Journal of Animal Science)*, vol. 27, no. 2, pp. 54–62, 2017.

[7] C. P. Suhendra, I. W. R. Widarta, and A. A. I. S. Wiadnyani, "Pengaruh Konsentrasi Etanol Terhadap Aktivitas Antioksidan Ekstrak Rimpang Ilalang (Imperata cylindrica (L) Beauv.) Pada Ekstraksi Menggunakan Gelombang Ultrasonik," *Jurnal Ilmu dan Teknologi Pangan (ITEPA)*, vol. 8, no. 1, p. 27, Mar. 2019, doi: 10.24843/itepa.2019.v08.i01.p04.

[8] L. Malangngi, M. Sangi, and J. Paendong, "Penentuan Kandungan Tanin dan Uji Aktivitas Antioksidan Ekstrak Biji Buah Alpukat (Persea americana Mill.)," *Jurnal MIPA*, vol. 1, no. 1, p. 5, Aug. 2012, doi: 10.35799/jm.1.1.2012.423.

[9] S. G. Dungir, D. G. Katja, and V. S. Kamu, "Aktivitas Antioksidan Ekstrak Fenolik dari Kulit Buah Manggis (Garcinia mangostana L.)," *Jurnal MIPA*, vol. 1, no. 1, p. 11, Aug. 2012, doi: 10.35799/jm.1.1.2012.424.

[10] K. Sridhar and A. L. Charles, "In Vitro Antioxidant Activity Of Kyoho Grape Extracts in DPPH and ABTS Assays: Estimation Methods for EC50 Using Advanced Statistical Programs," *Food Chem*, vol. 275, pp. 41–49, Mar. 2019, doi: 10.1016/J.FOODCHEM.2018.09.040.

[11] S. B. Nimse and D. Pal, "Free Radicals, Natural Antioxidants, and Their Reaction Mechanisms," *RSC Adv*, vol. 5, no. 35, pp. 27986–28006, 2015, doi: 10.1039/C4RA13315C.

[12] B. Ginting, M. Mustanir, H. Helwati, L. S. Desiyana, E. Eralisa, and R. Mujahid, "Antioxidant Activity of N-Hexane Extract Of Nutmeg Plants From South Aceh Province," *Jurnal Natural*, vol. 17, no. 1, p. 39, Mar. 2017, doi: 10.24815/jn.v17i1.6969.

[13] R. A. Day and A. L. Underwood, *Analisa Kimia Kuantitatif*, 6th ed. Indonesia: Erlangga, 2002.

[14] E. A. Maulana, I. A. R. Astiti Asih, and M. Arsa, "Isolasi dan Uji Aktivitas Antioksidan Senyawa Flavonoid dari Ekstrak Daun Jambu Biji Putih (Psidium guajava Linn)," *Jurnal Kimia*, Jan. 2016, doi: 10.24843/JCHEM.2016.v10.i01.p22.