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Isolation and Identification of Flavonoid Compounds from Mangrove Leaves Burus (*Bruguiera cylindrica (L.) Blume*)

Tonel Barus*, Jonathan Sihombing
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia
*Corresponding Author: tonel_barus@yahoo.co.id

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ABSTRACT

Flavonoid compounds have been successfully isolated from the Mangrove leave burus (*Bruguiera cylindrica (L.) Blume*) through a series of extraction processes. The maceration procedure, which involved soaking the material to be extracted in methanol solvent to allow the desired chemicals to dissolve gradually, was used for the first extraction. Following the addition of ethyl acetate, the extract was concentrated once more. This ethyl acetate extract is subsequently dissolved in methanol and partitioned with n-hexane. The obtained methanol layer was evaporated until dry, then analyzed using thin layer chromatography (TLC), with silica gel as the stationary phase and chloroform as the mobile phase. The composition of the chloroform:methanol eluent used varied (90:10, 80:20, and 70:30 v/v), and the pure amorphous solid compound with an orange-yellow color was successfully obtained using a 70:30 v/v eluent, weighing 16.3 mg and having an Rf value of 0.73. Compound identification was carried out using UV-Visible, FT-IR, and ^{1}H-NMR. Spectroscopic data indicate that the extracted compound belongs to the flavonol group.

Keywords: Daun Mangrove, Flavonoid, Flavonol, Isolation, Spectrocopy

ABSTRAK

Telah berhasil diisolasi senyawa flavonoid dari daun mangrove burus (*Bruguiera cylindrica (L.) Blume*) melalui serangkaian proses ekstraksi. Ekstraksi awal dilakukan menggunakan metode maserasi dengan pelarut metanol. Ekstrak tersebut dipekatkan, ditambahkan etil asetat, dan dipekatkan kembali. Ekstrak etil asetat ini selanjutnya dilarutkan dalam metanol dan diekstraksi secara partisi dengan n-heksan. Lapisan metanol yang diperoleh diuapkan hingga kering, lalu dianalisis menggunakan KLT fase diam silika gel dan fase gerak kloroform. Komposisi eluen kloroform:metanol yang digunakan bervariasi (90:10, 80:20, dan 70:30 v/v), dan senyawa murni berhasil diperoleh dengan eluen berbanding 70:30 v/v dalam bentuk padatan amorf berwarna oranye kekuningan dengan massa 16,3 mg dan nilai Rf sebesar 0,73. Identifikasi senyawa dilakukan menggunakan spektrofotometri UV-Vis, FT-IR, dan ^{1}H-NMR. Data tersebut mengindikasikan bahwa senyawa isolat merupakan golongan flavonol.

Kata Kunci: Daun Bakau Burus, Flavonoid, Flavonol, Isolasi, Spektroskopi

1. Introduction

Phytochemistry reveals that the extract of *Bruguiera cylindrica (L.) Blume* contains various types of compounds such as anthraquinones, terpenoids, flavonoids, saponins, phenolics, and alkaloids [1]. Flavonoids are compounds that make up about 60% of all natural polyphenols, found in all parts of plants, especially in flowers and leaves. The compound is known to have anticancer, anti-inflammatory, anticancer, anti-radioactive, and antioxidant properties [2]. Its antioxidant properties work by binding ions, inhibiting free radical-triggering enzymes, stimulating the body's antioxidants, and collaborating with other antioxidants to combat free radicals [3]

Flavonoid compounds are considered beneficial in food due to their antioxidant properties. The ability to neutralize oxidative species is very suitable to be combined with food, making it consumable for treating diseases such as cancer and heart disease [4]. The compound is commonly found in leaves or epidermal cells, which play a role in the physiological continuity of the plant.

Indonesia is known to have around 202 species of mangrove plants, consisting of 89 tree species, 5 palm species, 19 liana species, 44 epiphytes, and 1 type of fern. Mangrove forests easily host these plants, which include the genera Avicennia, Sonneratia, Ceriops, Bruguiera, and several species from the genus Rhizophora. Bruguiera, one of these genera, thrives in clay soil [5].

Mangrove plants have already been the subject of extensive research. Andianto et al. (2024) conducted one of the most significant studies between 2012 and 2014, discovering that compounds extracted from the leaves, bark, roots, hypocotyl, and flowers of *Bruguiera cylindrica (L.) Blume* can effectively kill mosquitoes [6]. Researchers have tested this plant's antibacterial activity against antibiotic-resistant bacteria and eye pathogens, demonstrating adequate antibacterial activity [7]. Egra et al. (2023) confirmed that the methanol extract from the leaves of *Bruguiera cylindrica (L.) Blume* can kill harmful bacteria and plant cells. This means that the extract can kill Vibrio that is harmful to plants [8]. Other research also confirms that *Bruguiera cylindrica (L.) Blume* serves as a natural antioxidant source and neutralizes free radicals through its flavonoid compounds [9].

Based on the above description, the aim of this work is obtaining flavonoid compounds from the leaves of *Bruguiera cylindrica (L.) Blume* using a combination of maceration extraction and partition extraction methods, followed by separation using column chromatography and further purification processes. Then the extraction results were identified using UV-vis, FT-IR, and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR).

2. Materials and Methods

2.1. Materials

The study used ^{1}H-NMR, FT-IR, UV-Visible, chromatography column, rotary evaporator, rotary evaporator flask, and UV lamp, burus mangrove leaves, methanol, ethyl acetate, n-hexane, Silica Gel 60, $FeCl_3$ 5%, Mg (s) + HCl (p), cotton, chloroform, Silica gel 60 F_{254} TLC plates.

2.2 Research Procedures

2.2.1 Sample Preparation

The leaves of the burus mangrove plant are cleaned and dried in a room with open air, then ground using a blender until 2500 g of mangrove leaf powder is obtained.

2.2.2 Phytochemical Screening of Flavonoid Compounds

A qualitative color reaction was used to identify fine dry burus mangrove leaf powder by adding 10 g of dried powder to an Erlenmeyer flask, adding 100 mL of ethyl acetate, and letting it stand before filtering. Three test tubes contained sample extract:

a. Tube 1: 3 drops of 5% $FeCl_3$ reagent were added, resulting in a change to black colloid.
b. Tube II: 0.1 mg of Mg powder and 3 drops of HCl were added, resulting in a pink solution.

2.2.3 Isolation of Flavonoid Compounds from Burus Mangrove Leaves

The leaves of *Bruguiera cylindrica (L.) Blume* that have been ground were weighed at 2500 grams, then macerated using 15 L of technical methanol for 48 hours. The results of the maceration were concentrated using a Heidolph rotary evaporator, then tested with 5% $FeCl_3$. The solvent is evaporated using a water bath, and tannin blocking is performed by dispersing the concentrated extract using ethyl acetate, then filtered. After a rotary evaporator evaporated and redissolved the filtrate in methanol, it was separated again with n-hexane until it was clear. From the n-hexane layer, the layer was evaporated again to produce 21.7 g of pure methanol extract.

The extract was analyzed using TLC with a stationary phase of silica gel 60 F_{254} E.Merck.Art 554 and an eluent of chloroform:methanol (90:10, 80:20, 70:30, and 60:40 (v/v)). Then the extract is applied to the activated TLC plate and placed into a saturated solvent mixture and eluted until a certain limit was reached. Next, the remaining eluate is dried and the spots are observed using UV light before being processed with 5% $FeCl_3$. The observed spots are then calculated for their Rf values. The fraction was then evaporated until an amorphous solid was formed. The same procedure was also followed for the chloroform:methanol mixtures (80:20, 70:30, and 60:40 (v/v)).

2.2.4 Identification of Isolated Compounds

The isolated compound was identified using three types of spectroscopy, namely UV-visible, FT-IR, and ^{1}H-NMR.

3. Results and Discussion

3.1 Isolation Results

The results of the phytochemical screening show that the leaves of the mangrove Bruguiera cylindrica (L.) Blume contain flavonoid compounds:

1. 5% $FeCl_3$ produces a black color
2. Mg(s)-HCl(p) produces a pink color

Fraction 38-112 produced an isolated compound in the form of an orange-yellow amorphous solid, weighing 16.3 mg. The TLC results, which used a chloroform:ethyl acetate eluent in ratios of 70:30, 40:60, and 30:70 (v/v), yielded Rf values of 0.73, 0.55, and 0.48 respectively.

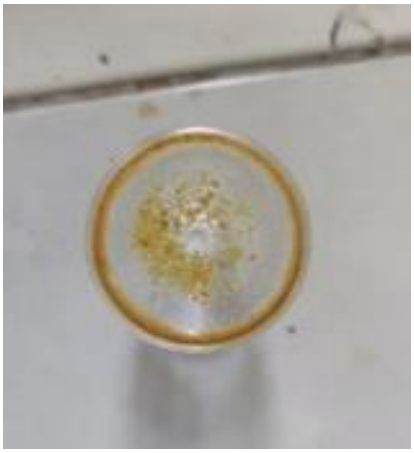

Figure 1. Isolated amorphous solid

3.2 UV-Visible Spectrum of Isolated Compounds

The UV-Visible spectrum of the isolated compound using methanol solvent is shown in Figure 2.

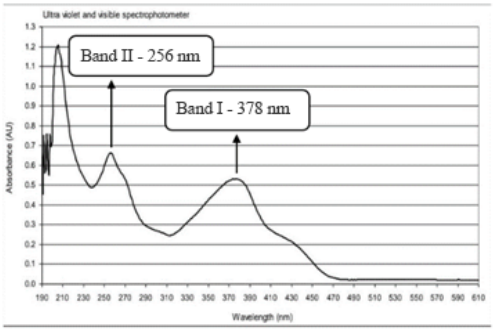

Figure 2. UV-Vis Spectrum of Isolated Compounds

UV-Vis analysis shows the presence of two maximum wavelength absorptions, as shown in the following Table 1.

Table 1. UV-Visible wavelength of isolated compounds

Peak	Wavelength (nm)	Absorbance
I	378	0.529
II	256	0.660

3.3 FT-IR Spectrum of Isolated Compounds

The FT-IR spectrum of the isolated compound using KBr pellets can be seen in Figure 3 below:

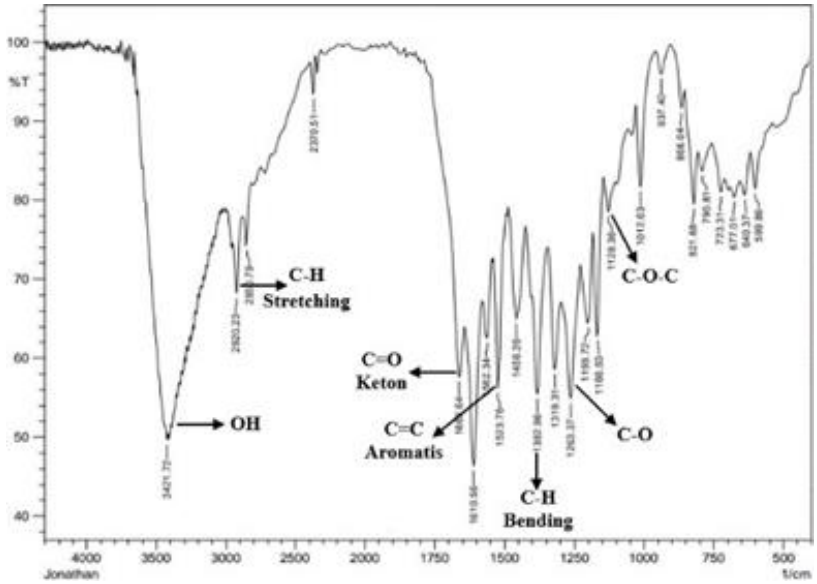

Figure 3. FT-IR spectrum of isolated compound

The FT-IR characterization results of the amorphous solid produced absorption bands in the wavenumber range listed in Table 2.

Table 2. Results of FT-IR spectrum analysis of isolated compounds number

Wavelenght (cm^{-1})	Wavelengh (cm^{-1}) (Pavia et al, 2001) dan (Harmita, 2009)	Vibration type	Functional group	Intensity
3421.72	3500-3200	Stretching	O-H	Sharp
2920.23	3000-2850	Stretching	C-H	Medium
1662.64	1725-1705	Stretching	C=O	Medium
1523.76	1600-1475	Stretching	C=C	Medium
1382.96	1450-1375	Stretching	C-H	Medium
1263.37	1300-1000	Stretching	C-O	Strong
1128.36	1070-1150	Stretching	C-O-C	Medium

3.4 Results of ^{1}H-NMR Analysis of Isolated Compounds

The results of the ^{1}H-NMR analysis of the isolated compound using methanol as the solvent and TMS as the internal standard:

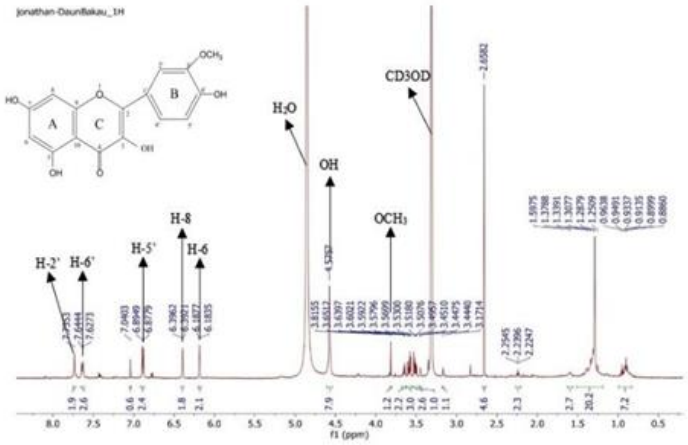

Figure 4. ^{1}H-NMR spectrum of isolated compound δ = 0.5 - 8.5 ppm

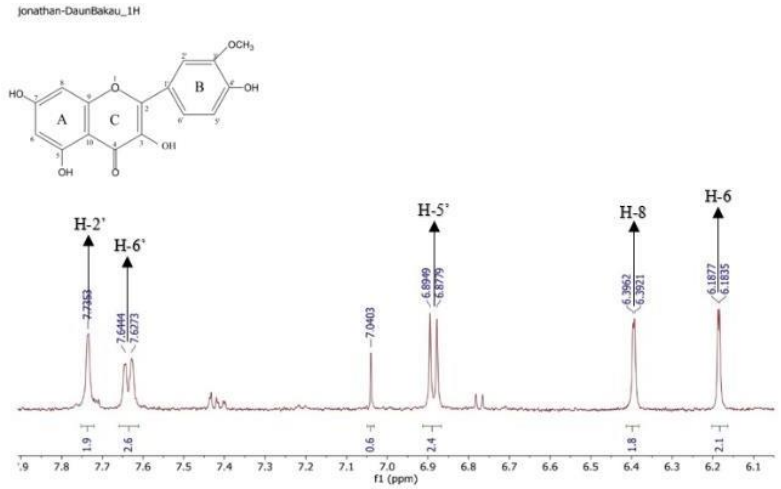

Figure 5. ^{1}H-NMR spectrum of isolated compound δ = 6.1 – 7.9 ppm

The analysis of the ^{1}H-NMR spectrum of the isolated compound has successfully identified various types of protons present in the molecule. The chemical shift data obtained from this spectrum has provided important clues regarding the chemical structure, such as the presence of certain functional groups or the types of chemical bonds formed. The complete analysis results were writen on Table 3.

Table 3. ^{1}H-NMR chemical shifts of isolated compounds

Atom (H)	δH of isolate (ppm)	Peak
H-2’	7.7353	Singlet
H-6’	7.6444-7.6273	Doublet
H-5’	6.8949-6.8779	Doublet
H-8	6.3962-6.3921	Doublet
H-6	6.1877-6.1835	Doublet
O-H	4.5757	Singlet
OCH_3	3.8155	Singlet

Based on the UV-Vis, FTIR, and ^{1}H-NMR spectra, the compound extracted from the mangrove leaves burus is a type of phenolic compound suspected to be methyl gallate (Figure 6).

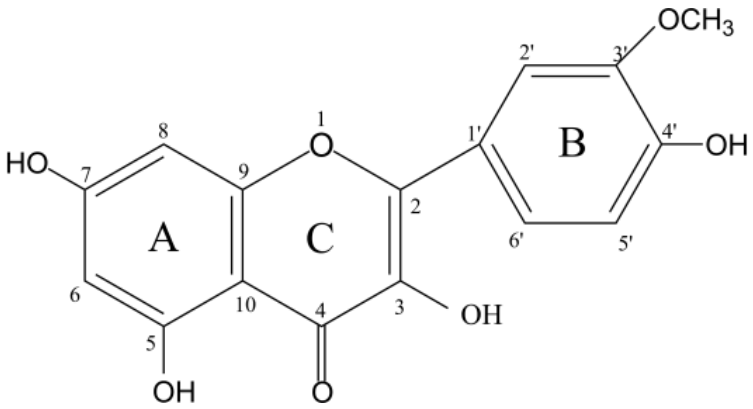

Figure 6. Structure of the isolated compound

4. Conclusion

The extraction of mangrove leave burus (*Bruguiera cylindrica (L.) Blume*) yields an amorphous solid with a yellowish-orange color, amounting to 16.3 mg (0.002608%). TLC analysis using eluent chloroform: methanol 70:30, 40:60, and 30:70 (v/v) each has an Rf value of approximately 0.73, 0.55, and 0.48, respectively. Phytochemical analysis of fraction 38-112 using the reagent Mg(S)+HCl(p) confirms the flavonoid chemicals found in Brus mangrove (Bruguiera cylindrica (L.) Blume). And the characterization results from UV-visible spectrophotometer, FT-IR, and ^{1}H-NMR reinforce the existence of the compound.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest.

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