Acta. Chim. Asiana., 2024, 7(1), 534-540
e-ISSN/p-ISSN 2550-0503/2550-049x
Available online at: www.aca.unram.ac.id


RESEARCH PAPER

# Characterization of methanol extract from Renggak (Amomum dealbatum Roxb) leaves and its application as an anion detector.

Najah Baroud[a], Syarifa Wahidah Al-Idrus[b], R. Rahmawati[b]*

[a]Department of Chemistry, University of Zawia, Libya

[b]Chemistry Education Division, University of Mataram, Indonesia

*E-mail: rahmawati_kimia@unram.ac.id

DOI: 10.29303/aca.v7i2.192

**Article info:**

Received 23/01/2024

Revised 03/10/2024

Accepted 05/10/2024

Available online 31/11/2024

**Abstract:** Renggak (Amomum dealbatum Roxb) shows significant potential as a natural resource with applications in various fields, particularly in environmental sensing. This capability positions Renggak as a promising candidate for developing eco-friendly chemosensors, contributing to improved monitoring of these critical anions in the environment and health. Additionally, its diverse phytochemical profile suggests potential uses in medicinal applications, further enhancing its value as a multifaceted plant. This research focuses on the characterization of the methanol extract from Renggak (Amomum dealbatum Roxb) leaves, and it's potential to detect iodide and phosphate anions. The extraction process involved soaking dried renggak leaves in methanol for 72 hours. Afterwards, the solution was filtered and evaporated, resulting in a paste-like extract. The extract was characterized using FTIR and GC-MS techniques, followed by testing its performance as a chemosensor. The paste extract was dissolved in methanol and mixed with a saturated solution of iodide and phosphate salts for the anion detection test. Then, 3 mL of each anion solution was combined with the extract, and any changes in the solution were observed. FTIR analysis revealed the presence of OH groups and C=O double bonds in the methanol extract. GC-MS analysis identified 20 compounds, with hexadecanoic acid and 9-octadecenoic acid being the most abundant. The extract demonstrated fluorescent properties in the anion detection test, acting as a chemosensor. When exposed to a UV lamp at 250 nm, the solution emitted a green fluorescent glow, indicating the successful detection of anions. These findings suggest that the methanol extract of renggak leaves could serve as an effective fluorescent chemosensor for iodide and phosphate anions.

**Keywords:** *anion test, iodide anion, phosphate anion, renggak leaf.*

**Citation:** Baroud, N., Al Idrus, S. W., & Rahmawati, R. (2024). Characterization of methanol extract from Renggak (Amomum dealbatum Roxb) leaves and its application as an anion detector. Acta Chimica Asiana, 7(2), 534–540. https://doi.org/10.29303/aca.v7i2.192

## INTRODUCTION

Amomum dealbatum Roxb is a wild plant belonging to the Zingiberaceae family. This plant lives in boggy places across water streams and hills [1–3]. This is an Indonesian endemic plant known by various names including "renggak" (Lombok), "wresah" (Java), "resak" (Malay), "ranggasa" (Sunda) and Java cardamon (English). Traditionally, Renggak has been used in Lombok, Indonesia, to prevent various diseases, including postpartum purification in females [4], septic abscesses [5], joint pain, antiseptic, muscular rheumatism, analgesia, loss of bowel movement, cough, and arthritis [6,7]. A recent study shows that reggae has anti-bacterial and anti-fungal activities [8].

Renggak (Amomum dealbatum Roxb) contains a wide variety of secondary metabolites. However, research on these metabolites is still limited. Hanifa et al. (2021) reported the presence of flavonoids, alkaloids, and tannins in Renggak [2]. A closely related plant, Amomum subulatum Roxb, possesses a diverse range of phytochemicals, including carbohydrates, amino acids, flavonoids, steroids, terpenoids, glycosides, tannins, alkaloids, and flavones, with 1,8-cineole and limonene as its main components [9,10]. These phytochemicals can serve as a reference for estimating the metabolite content in Renggak, given their shared genus (Amomum) and similar morphology. Many of these compounds have functional groups, such as hydroxyl and carbonyl, which can interact with anions through hydrogen bonding and charge interactions [11]. This property makes them potential agents for anion detection. Furthermore, oxyanions like phosphate are linked to metabolic processes, suggesting they may react with the metabolites produced by Renggak. In addition, oxyanions such as phosphate correlate with metabolic processes that should be reactive to the metabolite product [12].

Several anions, such as phosphate and Iodide, play essential roles in human, animal and plant health and metabolism [13–18]. However, excessive intake of both anions can endanger human health and the environment. Iodide is essential for human growth, thyroid function, neurological growth activity, and brain function [19]. Accumulation of Iodide in the human body can cause adverse effects, while phosphate is responsible for carcinogenesis and eutrophication of rivers and lakes [19–22]. Iodide and phosphate anions are also essential components in the chemical industry, fertilizers, organic synthesis, and biological processes, so the use and release of iodide and phosphate anions into the environment continue to rise [23–26]. It is feared that environmental pollution caused by iodide and phosphate will contaminate drinking water sources and sediments. Therefore, detecting and monitoring iodide and phosphate anions is necessary and urgent. Sensitive, simple, and facile methods such as chemosensors are needed to detect these anions.

Most chemosensors today are synthetic compounds and contain metal as the chemosensor core. The synthesis process and use of metal as a chemosensor can potentially cause new problems for health and the environment. The analysis process must also use sophisticated instruments to determine the results. In this study, reggae extract, as a natural, low-cost, and non-toxic chemosensor, was used as a colourimetric sensor for the analysis of phosphate and iodide ions in water and soil. The affluence of pH, reaction time, and reggae extract concentration were studied to optimize the chemosensor's selectivity and sensitivity.

Several chemosensor compounds have been made from vanillin via synthetic pathways. The chemosensor compounds produced were the benzimidazole group and its derivatives, the benzoxazole group and its derivatives, and the flavon group. Among them are compounds A Novel 4-(1H-Benzimidazol-2-yl)-2-ethoxy-phenol Derived Fluorescent Sensor for Determination of CN Ion [27], A Naked-Eye Colorimetric Receptor for Anions Based on Nitro Group Featuring with Benzimidazole Unit [28], A 4(1H-Benzo[d]oxazole-2-yl)-2-methoxyphenol as dual selective sensor for cyanide ion detection [29], Synthesis of 5-Nitrovanillin in Low Temperature as Cyanide Anion Sensor [30],

This study aims to analyze the compound composition of methanol extracts from Renggak leaves and evaluate its effectiveness as a chemosensor for detecting phosphate and iodide anions. The results are expected to provide insights into the specific compounds in Renggak leaves and establish their capacity for anion detection. Ultimately, this research could lead to the development of a novel, eco-friendly chemosensor, enhancing methods for monitoring these anions in both environmental and health-related applications.

## MATERIALS AND METHODS

### Renggak Extraction

The extraction method utilized in this study follows common techniques used for extracting compounds from natural materials (figure 1). The materials used, such as methanol, were of analytical grade (Sigma Aldrich, Singapore). First, 100 grams of powdered Renggak leaves were placed in a jar and soaked in 500 mL of methanol for 24 hours. The following day, the mixture was filtered, and the filtrate was soaked in methanol for another 24 hours before being filtered again. The combined filtrate was then evaporated using an evaporator until a thick liquid extract of the coarse Renggak leaves was obtained. This extract was air-dried to remove any remaining solvent, resulting in a dry paste. Characterization of the crude extract was conducted using FTIR and GC-MS techniques.

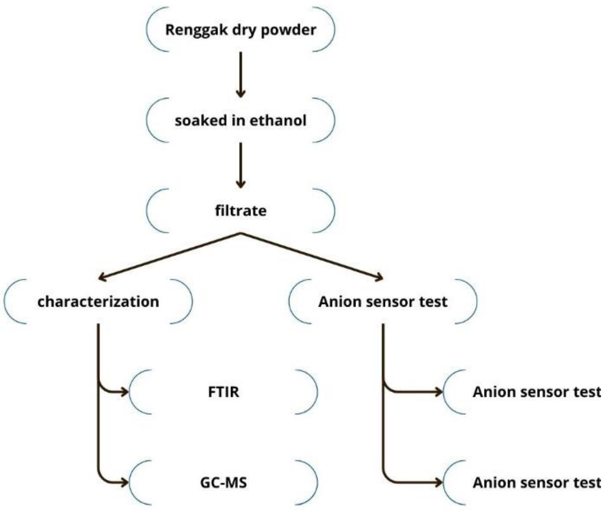


Figure 1. Experimental outline

### *Anion test*

A total of 1 gram of reggae leaf extract paste was dissolved in 25 mL of methanol, and then 5 ml of each solution was put into 3 different vials. On the other hand, a saturated solution of NaI and phosphate salt was made. Then, 5 drops of the saturated salt solution were added to the vial containing the reggae extract solution. Then, observe the colour changes that occur under a UV lamp.

## RESULTS AND DISCUSSION

### Renggak Extraction

Renggak leaves that are macerated using methanol produce a dark green extract (Figure 1). The polar solvent methanol will dissolve polar compounds from renggak leaves.

Characterization of the compound content from the extraction of renggak plant leaves with methanol solvent was determined using FTIR and GCMS instruments.

Figure 2 Methanol extraction result of Renggak leaf

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### FTIR Instrument Result

The FTIR spectra results show several important peaks emerging from the methanol extract. The wide peak at 3400.26 $cm^{-1}$ corresponding to the stretching vibration of the hydroxyl group [1], the OH stretching band regions [2] indicates the presence of an –OH group, although this area is also an absorption area for the –NH group, but because there is no strong peak appearing in the 1180-1360 area which is the absorption area for the amine/amide group, it shows that the compound does not contain a -NH group. The OH group that appears can come from alcohol, ether, carboxylic acid, or ester because the appearance of a strong peak in the 1253.02 $cm^{-1}$ area is confirmed again by the appearance of a strong peak in the 1077.97 $cm^{-1}$ area. The study of the O–H stretch mode is prompted by the strong correlation between the frequency of O–H stretch vibration and the individual hydrogen-bond strength [3,4].

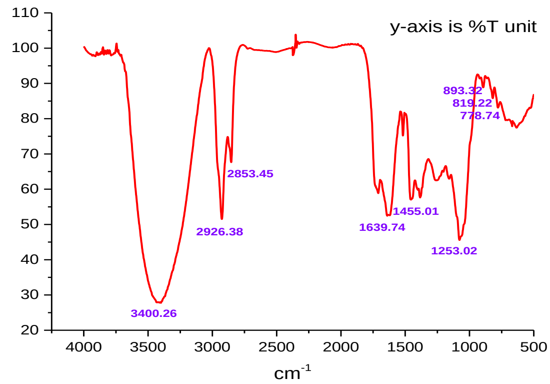



Figure 4. FTIR spectra of methanol extract of Renggak leaf.

The changing peak in the 1455.01 area indicates the absorption of aromatic C=C, and 3 sharp peaks in the 893.31 $cm^{-1}$, 819.22 $cm^{-1}$, and 778.74 $cm^{-1}$ areas indicate the stretching of the aromatic C-H bond.

**GC-MS Instrument Result**

From the GC/MS results, chromatogram retention time data will be obtained with several compound peaks (the largest abundance can be seen from the highest graph). From the spectrogram data, the fragmentation pattern of each compound was obtained. The structure of each can be known based on the characteristic fragmentation patterns and basic peaks.

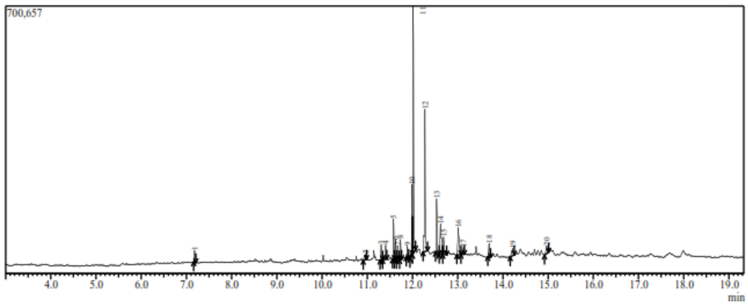



Figure 5. GCMS spectra of methanol extract of Renggak leaf.

GC-MS spectra of methanol extract showed 20 peaks with varying abundances with RT ranging from 7,180 min to 14,970 min. The discussion this time is limited to compounds in the peak with the highest abundance and percentage (31.71%), peak 11, where peak 11 refers to hexadecanoic acid or palmitic acid.

**Mass Spectroscopy Analysis**

Interpretation on mass-spectrum GC-MS was conducted using the database of the National Institute of Standards and Technology (NIST) [5–11]. Having more than 62,000 patterns. The unknown components' spectrum was compared with those of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained [12].

**a) Peak 11.**

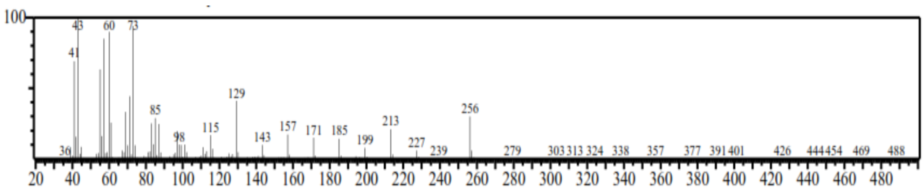



Figure 3. Peak 11's mass spectra

**a) Peak 12.**

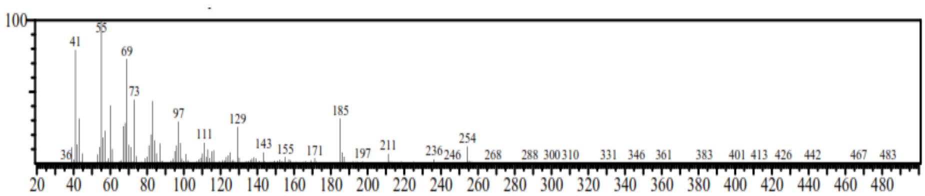
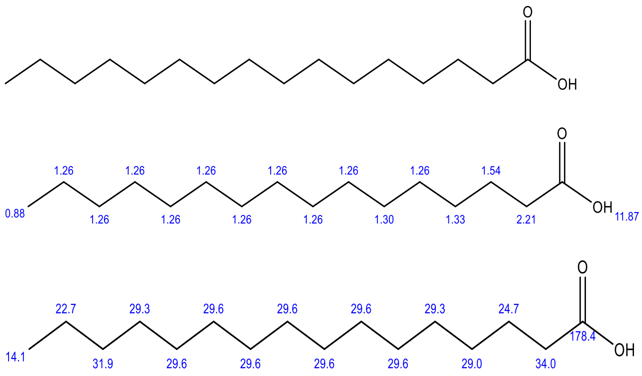



Figure 5. Peak 12's mass spectra

The compound at peak 11, which shows m/z 256, RT 12.015, is a hexadecanoic acid compound with the number of atoms C: 16, H: 32, O: 2 and formula $C_{16}H_{32}O_2$.

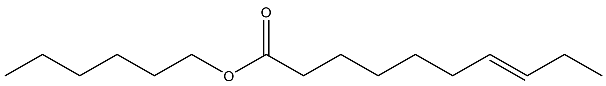
Properties prediction of peak 11 (by ChemDraw application):



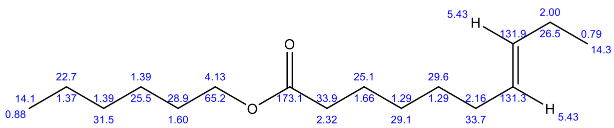
Exact Mass: 256.24; Molecular Weight: 256.43; m/z: 256.24 (100%), 257.24 (12.3%), 258.25 (1.4%). Elemental Analysis: C, 74.94; H, 12.58; O, 12.48

Figure 4. Theoretical analysis of Peak 11's mass spectra: a) mass spectra; b) $^{1}$H-NMR prediction; c) $^{13}$C-NMR prediction.

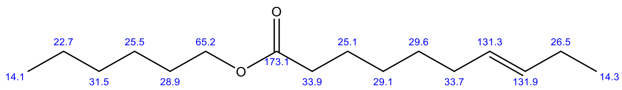
The compound at peak 11, which shows m/z 254, RT 12.015, is a 9-octadecenoic acid compound with the number of atoms C: 18, H: 34, O: 2 and formula: $C_{18}H_{34}O_2$.



a. Structure prediction of peak 12 compounds (based on mass spectra)



b.



c.

Exact Mass: 254.22; Molecular Weight: 254.41; Elemental Analysis: C, 75.54; H, 11,89; O, 12.58; chemical formula: $C_{16}H_{30}O_2$

Figure 6. Theoretical analysis of Peak 12's mass spectra; a) mass spectra; b) $^{1}$H-NMR prediction; c) $^{13}$C-NMR prediction.

**Anion Test On The Entire Crude Extract Of The Renggak Leaves**

Four variations were obtained from crude extracts from renggak leaves and fruit, namely crude ethanol extract from leaves (EEL) and fruit (EEF), crude methanol extract from leaves (EML) and fruit (EMF). Testing the activity of crude extract as an anion chemosensor, with 2 types of anions from salt: NaI (ion $I^-$) and $NaH_2PO_4$ (ion $H_2PO_4^-$), the result shown in Table 1.

**Table 1. Chemosensor Test Result**

| | **ion $I^-$** | **ion $H_2PO_4^-$** | **Explanation** |
|---|---|---|---|
| EEL | + | + | Fluorescence off-on green, no colour |
| EML | + | + | Fluorescence off-on green, no colour |
| EEF | - | - | no fluorescence, no colour |
| EMF | - | - | no fluorescence, no colour |

Table 1 shows that the crude methanol extract from reggae leaves gave a positive response to iodine anions and phosphate anions, namely in the form of changes in fluorescein (if observed under a λ 250 nm UV lamp), with a light green fluorescent colour.

The following discussion is limited to the anion test results from the methanol extract of renggak leaves. Figure 3 shows the response of crude methanol extract to iodine anions [13–17] and phosphate [18,19] in the form of changes in fluorescence when observed under a UV lamp. The fluorescent type is 'off-on', In addition to the emission of bright green fluorescence under UV lamp [20].

Phosphate is a fundamental building block of living systems, such as nucleic acids, nucleotides, and nucleosides, and plays a crucial role in many biochemical processes. It is known that phosphate exists in both $H_2PO_4^-$ and $HPO_4^{2-}$; meanwhile, Iodide is a pollutant in drinking water [21].

EML + no anion presence (no fluorescence and no colour)

EML + anion under UV lamp (fluorescence on with green colour, no colour)

Figure 6. Crude methanol extract from renggak leaves, without anions (top) and with anions (bottom): left) blanko, mid) with $I^-$ anions, right) with $HPO_4^{2-}$ anions.

## CONCLUSION

The methanol extract from Renggak leaves demonstrates effectiveness as a chemosensor for $I^-$ and $HPO_4^{2-}$ anions. It is an environmentally friendly sensor for detecting iodide and phosphate anions derived from natural sources. GC-MS characterization of the crude extract revealed two key compounds, hexadecanoic acid and 9-octadecenoic acid.

## ACKNOWLEDGEMENTS

This research was carried out with funding from DIPA BLU LPPM Mataram University no. 2534/UN18.L1/PP/2023.

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