Study of biomedical properties of *Rhizophora mucronata* fruit from Rembang, Central Java

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**Abstract.** Indonesian in coastal region utilize mangrove ecosystem for aquaculture, fishing ground, firewood source, tourism, nevertheless it is rarely reported as a source of biomedical substances. *Rhizophora mucronata* is one of the most common mangrove species in Rembang, Central Java. However, study of biomedical prospect of its fruit is neglected. This study aimed to investigate the antimicrobial activity of *R. mucronata* fruit extract to inhibit human pathogens (*E. coli*, *S. aureus*, *M. luteus*, *T. rubrum*, and *C. albicans*), as well as its anticancer activity against P388 murine leukemia cells. Metabolite profile was characterized through phytochemical test and HPLC. The result showed there was no antimicrobial activity of *R. mucronata* fruit extract against all human pathogens. Cytotoxic assay indicated a moderate anticancer potential with IC$_{50}$ value of 398 µg/mL. Phytochemical test indicated the presence of alkaloid, flavonoid, and steroid/triterpenoid in the crude extract.

1. Introduction

Mangrove as a coastal plant is exposed by extreme environmental stresses such as salinity, tidal, UV, temperature, flood, etc [1]. The conditions induce production of secondary metabolites in mangrove to help themselves to survive during the exposure of abiotic and biotic stresses [1,2]. Previous studies reported that mangroves produce phenolic derivatives such as flavonoid, flavone and flavonol, as well as terpenoid derivatives such as polyisoprenoid as a response of environmental stresses [1–4]. In addition, those secondary metabolites are expected to have biological activity for human health.

*Rhizophora mucronata* is widely distributed mangrove in Indonesia. In biotechnological prospection, this species is regularly reported as a producer of secondary metabolites with outstanding biomedical properties [5]. Sumardi *et al.* [6] reported *R. mucronata* leaves showed antibacterial...
activity, whereas Sari et al. [7] stated that R. mucronata leaves also had anticancer property against WiDr cell line. This potential is prospectively applied to cure cancer and infection of multi-drug resistant organisms (MDRO) which are two global health problems nowadays [8,9]. In addition, our previous study also reported an anticancer activity of R. mucronata leaves methanol extract against P388 murine leukaemia cancer cells [10].

Even though there are plenty researches discovered biological activity of R. mucronata, most of the studies worked with the leaves [11–14]. On the other hand, study of biomedical properties from R. mucronata fruit is less reported. Furthermore, different and unique biotic and abiotic stresses in every location are suggested to induce the production of diverse or even specific secondary metabolites in plant [15,16]. Therefore, our study focused on metabolites profiling of R. mucronata fruit from Rembang, Central Java which never been reported and screening of its biomedical properties against multi-drug (MDR) bacteria, clinical pathogenic fungi and cytotoxicity against P388 murine leukaemia cells.

2. Methodology
2.1 Sampling and sample preparation
Fresh R. mucronata fruits were collected from Rembang, Central Java (Fig. 1) then kept in a cool box. Samples were transferred to Natural Product Laboratory, Universitas Diponegoro for extraction. Fresh fruits were cleaned using water then sun-dried.

![Figure 1. Sampling location at Rembang Mangrove Forest Ecopark, Central Java](image)

2.2 Metabolite extraction
The dried fruit was resized by using blender, then the semi powder samples were extracted using methanol. Maceration method with agitation using shaker at 115 r.p.m. for 24 h was carried out. Then, the organic solvent was separated from the samples. Organic solvent then was evaporated by using rotary-evaporator at 30-35 °C. The semi-solid crude extract was weighed then used for the further analysis.

2.3 Phytochemical test
A basic secondary metabolites profiling was done by using phytochemical test. Five secondary metabolite groups which consisted of alkaloid, flavonoid, glycoside, saponin, and steroid/triterpenoid were analyses qualitatively according to Sibero et al. [10].

2.4 HPLC analysis
Crude extract of *R. mucronata* fruit was dissolved in DMSO with concentration 1 mg mL\(^{-1}\). The liquid extract then was filtered and injected into high performance liquid chromatography with diode array detector (HPLC-DAD). Formic acid buffer 0.1% (Wako, Japan) and acetonitrile (Wako, Japan) were carried out as mobile phase, while COSMOSIL 3C18-AR-II (4.6ID × 100 mm) from Nacalai Tesque was chosen as the column. HPLC analysis protocol was carried out according to our previous report [10].

2.5 Antimicrobial activity assay
Multi-drug resistant (MDR) bacteria such as *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus* and clinical pathogenic fungi such as *Candida albicans* and *Trichophyton rubrum* from Dr. Kariadi Hospital, Semarang were used as target pathogens. MDR bacteria were revived and cultured on Mueller Hinton (MH, Difco) while Sabouraud Dextrose (SD, Difco) were utilized to revive and culture the pathogenic fungi. Crude extract was diluted in DMSO to obtain concentration 1, 0.5, 0.25, and 0.125 mg mL\(^{-1}\). In total 10 µL of each crude extract was transferred into 96-well microplate which contained 150 µL broth media (MH for bacteria and SD for fungi) and 40 µL of bacterial stock (0.5 McFarland) then mixed gently using micropipette. The mixture was incubated for 24 h at 37 ºC, then 50 µL of WST-1 (Roche) was added into each well then re-incubated for 2h at 37 ºC. The presence of yellow colour indicated the living cells while transparent well indicated the inhibition of microbial growth.

2.6 Cytotoxicity assay
Cytotoxicity potential of *R. mucronata* fruit extract was tested against P388 Murine leukaemia cancer cells. The protocol of this assay has been published at Sibero et al. [10].

3. Result and discussion
The selection of *R. mucronata* as the sample in this study based on the dominancy of this species at Rembang mangrove forest ecopark. The *R. mucronata* at sampling location was noted as the result of a successful rehabilitation and plantation activity. Saputro et al. [17] noted that *R. mucronata* density at Rembang mangrove forest ecopark was 0.2-0.32 ind m\(^{-2}\). They also noted the Rembang mangrove forest ecopark had highest tidal (1.1 m) at 6 a.m. and the lowest tidal (0.53 m) at 12 p.m. Moreover, in the location, various associated organisms were also found. These biotic add abiotic stresses are suspected to induce secondary metabolite production in *R. mucronata*. In this study, the samples produced 1.25% crude extract with sticky-solid form and deep brown colour. The presence of bioactive compounds from the crude extract is presented by Table 1.

The result of bioactive detection through phytochemical test (Table 1) showed the presence of alkaloid, flavonoid and steroid/triterpenoid in the crude extract. Purwaningsih et al. [18] extracted the metabolites of *R. mucronata* fruit from Seribu Island, Jakarta with ethanol and detected the presence of flavonoid, steroid/triterpenoid and saponin. On the other hand, Podungge et al. [19] stated that the methanol extract of *R. mucronata* fruit gave positive result on detection of flavonoid, triterpenoid, tannin and saponin. The various results of phytochemical test for plant sample is strongly correlated with the organic solvent that used to extract the metabolites and the presence of bioactive compounds in the samples. The result of HPLC-DAD analysis of the crude sample is presented by Figure 2.
Table 1. Bioactive content of R. mucronata fruit extract

| Bioactive Groups   | Indicator                                                                 | Documentation                | Result  |
|--------------------|---------------------------------------------------------------------------|------------------------------|---------|
| Alkaloid           | A formation of yellow to orange precipitates after addition of Dragendorff reagent | Picture of yellow precipitate | Detected|
| Flavonoid          | A presence of yellow/orange/reddish color in amyl alcohol layer            | Picture of color in layer    | Detected|
| Saponin            | A stable foam after 30 min and addition of 2N HCl                         | Picture of foam              | Not Detected|
| Steroid/Triterpenoid| A formation of green coloration of the upper layer and red coloration of the lower layer | Picture of color in layer    | Detected|
| Glycoside          | A presence of brown ring between the layers                                | Picture of brown ring        | Not Detected|

Figure 2. Chromatogram of R. mucronata fruit extract which detected using DAD at 254 nm

The methanol crude extract of R. mucronata fruit only contained three major peaks when detected using DAD at 254 nm (Figure 2). The first peak appeared at 7.8 min with maximum UV absorption at 232, 270, 284 and 354 nm; the second peak appeared at 8.8 min with maximum UV absorption at 234, 280 and 352 nm; while the last peak appeared at 10.9 min with maximum UV absorption at 244 and 280 nm. These peaks were not suitable with any data in our computer data base therefore, it is really suggested to isolate these peaks in the further works. In order to understand the biomedical properties, anti-MDR bacteria, antifungal and anticancer were conducted. The result of antimicrobial assay is presented by Figure 3 while cytotoxicity by Figure 4.

Most of R. mucronata bioprospecting studies reported the biological activity from leaf and barks. Crude extract of R. mucronata leaf showed antimicrobial activity against B. subtilis, E. coli, K. pneumonia, S. aureus, Aspergillus niger, Acremonium sp. and Penicillium digitatum [6,20]. Further,
the bark extract also had antimicrobial activity against *S. aureus*, *S. epidermidis*, *E. coli*, *Salmonella paratyphi*, *Shigella flexneri*, *S. boydii*, and *S. dysenteriae* [21]. Figure 3 shows all wells had deep-yellow colour. Unfortunately, the yellow colour in the wells indicated that the bacterial growth was not inhibited, therefore it means the *R. mucronata* fruit extract was not a prospective agent against anti-MDR bacteria neither anti-clinical pathogenic fungi (MIC > 1 mg mL\(^{-1}\)). The study which was conducted by Nurdiani *et al.* [22] reported the ability of *R. mucronata* fruit extract inhibited the growth of non-MDR *E. coli* and *S. aureus* while in our current study, MDR and clinical pathogens were carried out. The absence of antimicrobial activity of the fruit crude extract might be impacted by the antimicrobial sensitivity of the pathogens. MDR pathogens have been known to have more resistance to various antimicrobial agents [9], therefore the secondary metabolites from the fruit extract did not give any effect to the pathogens.

![Figure 3](image3.png)

**Figure 3.** Result of antimicrobial assay of *R. mucronata* fruit extract

(A. *E. coli*, B. *S. aureus*, C. *M. luteus*, D. *C. albicans*, E. *T. rubrum*)

Anticancer of *R. mucronata* leaf has been widely reported. Sari *et al.* [7] stated that the polyisoprenoids from the leaf had cytotoxic effects to the human colon cancer cell line WiDr via apoptosis mechanism, while Palaniyandi *et al.* [23] stated that squalene from the leaf gave antigastric carcinogenic effect at 50 μg/mL in AGS cell lines. In addition, our previous study also obtained the
anticancer potential of the leaf crude extract [10]. In this current study, it is highlighted that crude extract of *R. mucronata* fruit also showed anticancer property with moderate toxicity at IC<sub>50</sub> value of 398 µg mL<sup>-1</sup> against P388 murine leukaemia cells. The ability of this crude extract in inhibiting the cancer cell is expected as the result of the presence of terpenoids compounds. As mention before, several derivatives of terpenoids such as polysoprenoids and squalene have an important role in inducing apoptosis of cancer cells [7,23]. Furthermore, this study suggests the *R. mucronata* fruit extract as a potential source of anticancer agent against P388 murine leukaemia cancer cells. However, to confirm this hypothesis it is also strongly suggested to isolate three major peaks that detected by HPLC-DAD (Figure 2), because one of them or even all of them are might be the lead compounds for anticancer agent.

4. Conclusion

The *R. mucronata* fruit extract from Rembang, Central Java contained alkaloid, flavonoid and steroid/triterpenoid. The HPLC-DAD chromatogram indicated the presence of three major peaks at 254 nm. Unfortunately, bioassays result indicated the inability of *R. mucronata* fruit extract inhibit the MDR bacteria and clinical pathogenic fungi. However, the cytotoxic assay showed anticancer potential of the crude extract against P388 murine leukaemia cancer cells with IC<sub>50</sub> value of 398 µg mL<sup>-1</sup>. Further, the presence of terpenoids and three major peaks at the HPLC-DAD chromatogram was suggested to have an important role to the anticancer property of the crude extract.

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**Acknowledgement**

The authors would like to thank the Faculty of Fisheries and Marine Science, Universitas Diponegoro for the research funding with contract number 023/UN.7.5.10.2/PP/2020 and to Biotechnology Research Centre, Toyama Prefectural University, Japan to provide cancer cells, reagents and HPLC to conduct the study.