The 2nd International Conference On Indigenous Knowledge For Sustainable Agriculture 2023
ISBN : 978-623-161-062-1

Exploration of Rhizophoraceae against *Ralstonia solanacearum* as Natural Pesticides

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ABSTRACT

The Rhizophoraceae family dominates the mangrove forest. The mangroves employed in this study were found in Tarakan city, North Kalimantan, in the mangrove and proboscis monkey conservation area. The aim of this research is to identify mangrove species that have the potential to be natural pesticides against wilt disease in plants. The following mangroves were used in this study *Bruguiera parviflora* (Roxb.) Wight & Arn. ex-Griff., *Bruguiera cylindrica* (L.) Blume, *Ceriops tagal* (Perr.) C.B.Rob., *Rhizophora apiculata* Blume, *Rhizophora mucronata* Poir. The three parts of the samples were extracted leaf, wood and bark with n-hexane, ethyl acetate and Methanol. The agar-well method was employed in this investigation against *Ralstonia solanacearum*. The ethyl acetate extract of the stems of B. parviflora had the highest activity of 57.7% at a concentration of 2000 ppm, while the ethyl acetate extract of the stems of *B. cylindrica* had the highest activity of 45% at a concentration of 2000 ppm among the five mangrove plants. More research is needed to obtain pure chemicals that has inhibition to the bacteria.

Keywords:

Antimicrobial, Conservation area, Natural pesticide, *R. solanacearum*, Tarakan island

INTRODUCTION

Mangrove forests are sensitive to tides since they are positioned on the sea's edge in brackish seas. Mangrove forests are renowned as a barrier against the onslaught of waves; they are also known as a habitat for marine biota, a site for puddling, and a supply of organic materials. Mangrove forests are made up of plants that have adapted and evolved to survive, grow, and thrive in areas with significant soil salinity. Mangrove forests can be found all along the equator, in both the tropics and to a lesser extent in the subtropics [1].

ISBN : 978-623-161-062-1
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In mangrove forests, the Rhizophoraceae family predominates. Australia, Papua New Guinea, Indonesia, India, East and West Africa, and areas of Latin America are all home to this family. This tree and shrub family includes 16 genera and 120 species [2]. *Bruguiera parviflora* (Roxb.) Wight & Arn. ex-Griff., *Bruguiera cylindrica* (L.) Blume, *Ceriops tagal* (Perr.) C.B.Rob., *Rhizophora apiculata* Blume, and *Rhizophora mucronata* Poir were employed in this work. These five plants are the most common in conservation zones for mangroves and proboscis monkeys [3].

The public's use of mangrove plants is still quite limited, owing to the lack of information supplied by this plant type. The aim of this research was to use five different varieties of mangroves to limit the growth of gramme-negative bacteria that induce wilting in plants of the solanacearum family, specifically *Ralstonia solanacearum*[4][5]. This bacterium lives in the soil, infects the plant from within, and then enters the plant's xylem ducts [6]. Minerals from the soil cannot be transported to the leaves unless bacteria accumulate and fill the channels. This is what causes plants to wilt as a result of a lack of transpiration. Granville Wither is the name for this. Typically, *R. solanacearum* infects tomato, pepper, aubergine and potato plants [7]. Information The ability to inhibit plant wilt bacteria originating from mangrove plants is something new, and there has been no previous research, so this research is very important to do to get the latest information about the role of mangrove plants in inhibiting the growth of *R. solanacearum*, which causes wilt disease in agricultural crops.

METHOD

Plants and chemical substances

The Mangrove and Proboscis Monkey Conservation Area in Tarakan City, North Kalimantan, Indonesia, provided five mangrove plants. Merck (Darmstadt, Germany) provided the chemical components, mainly glucose and nutritional agar. Other chemical ingredients of high grade and purity were obtained commercially, along with HPLC solvent. The plant herbs utilised were kept in the Laboratory of Dendrology at Universitas Mulawarman's Faculty of Forestry.

Extraction

Extraction of plants part are leaves, bark, and wood of Rhizophora species were collected in the field, with each sample weighing 250–500 g. The samples were air-dried at room temperature for three days before being serially macerated with n-hexane and ethyl acetate and shaken (IKA-KS 260-C shaker, Switzerland). After that, the samples were filtered using Whatman No. 2 paper (Maidstone, UK). Finally, using a rotary evaporator set to 35–40 °C, the solvent was removed from the extracts. The extracted samples were dried in a Memmert 100–80 vacuum oven. These plants from previous studies [3]

Antibacterial Assay

The antibacterial assay was carried out using the diffusion method described by Egra, [5] with minor modifications. *R. solanacearum* was cultivated on nutrient agar as the medium in this study. By adding 20 mL of distilled water mixed

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with agar media to the Petri dish, media were created. The bacteria were then disseminated across the medium surface at 20 L until uniformly distributed in areas of the surface perforated at 7 mm. At a concentration of 100 g/mL, chloramphenicol was utilised as a positive control[8].

RESULT AND DISCUSSION

Mangrove forests have an extraordinary wealth of plants. The mangrove and proboscis monkey conservation area is the only one in Indonesia that is located very close to the city centre. Only one to five minutes of mileage of Tarakan city. With an area of 22 ha. This area is covered with 22 types of plants and more than 50 monkeys, including proboscis monkeys. In addition, this mangrove forest is a habitat for other biotas such as birds, crabs, hermit crabs, fish, shrimp, turtles, and lizards. The KKMB mangrove forest is dominated by the Rhizophoraceae family. This article contains follow-up research from previous

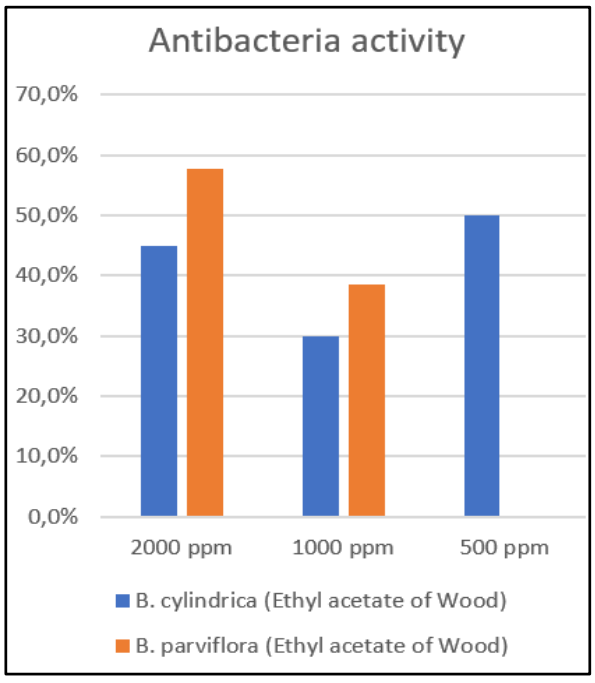

Figure 1. Antibaterial activity of Mangrove plant against *R. solanacearum*

research in our work [3]. In this article, we present only antibacterial data against *R. solanacearum*. This bacterium is the main cause of the wilting of agricultural crops such as eggplants, tomatoes, potatoes, and other solanacearum families [9].

This study tested 5 mangrove plants from the Rhizophoraceae family, on 3 plant parts namely, leaves, bark, and wood. The content of secondary metabolites in plant parts has potential. The potential for inhibition against bacteria is also different. The three parts of the plant were extracted using three solvents namely, hexane, ethyl acetate, and methanol. The level of polarity in plant compounds helps separate groups of compounds based on their polarity, and makes it easier to identify the phytochemicals in these plants, phytochemical tests have been carried out by our previous research [3]. The level of polarity in plant compounds helps separate groups of compounds based on their polarity and makes it easier to identify the phytochemicals in these plants. Phytochemical tests have been carried out in our previous research [10]. This research resulted in the inhibition ability of the five types of mangrove plants not being significant and tending to have no inhibition at all except for two samples, namely the ethyl acetate extract of *B. parvifolia* wood and the ethyl acetate extract of *B. cylindrica* wood.

This study demonstrated the ability of *B. cylindrica* wood parts in ethyl acetate solvent to inhibit bacteria up to 45%, 30%, and 50% at concentrations of 2000, 1000, and 500 ppm,

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respectively. Whereas in *B. parvifolia*, the wood in the ethyl acetate solvent was able to inhibit higher concentrations up to 58% and 38% with concentrations of 2000 ppm and 1000 ppm, respectively (Figure 1).

The phytochemical content of these two plants has in common that there are alkaloids, but in *B. parviflora*, there are flavonoids. Alkaloids and flavonoids are known as inhibitors of bacterial growth. The mechanism of action of flavonoids as antibacterial compounds is divided into three categories: inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism. In inhibiting the synthesis of nucleic acids, the A and B rings of flavonoid compounds play an important role in the intercalation or hydrogen bonding process by accumulating nucleic acid bases, thereby inhibiting the formation of DNA and RNA. The results of the interaction of flavonoids will also cause damage to the permeability of the cell wall [11]. In inhibiting the function of cell membranes, flavonoids will form complex compounds from extracellular and dissolved proteins so that cell membranes will be damaged and intracellular compounds will come out. Whereas in inhibiting energy metabolism by inhibiting the use of oxygen by bacteria, namely by preventing the formation of energy in the cytoplasmic membrane and inhibiting bacterial motility, which plays a role in antimicrobial activity and extracellular proteins [12]. Wood extracts from *B. parvifolia* and *B. cylindrica* have a strong ability to inhibit the growth of *R. solanacearum*.

CONCLUSION

The antibacterial action of *B. cylindrica* against *R. solanacearum* is significantly contributed to by the presence of alkaloids in this species. This research needs to be investigated in greater depth in order to provide conclusive evidence that natural substances can be utilised in the production of natural pesticides.

ACKNOWLEDGMENTS

Thank you to the Research and Community Service, Borneo Tarakan University for funding this research, Thank you to the KKMB management and the North Kalimantan Forestry Department. thank you for togetherness and friendship in completing this writing to all the UBT Campus Mosque Marbot Team, and all Kindness Entities in the Tarakan City.

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