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Phytochemical characteristics and Antimicrobial activity of medical plant *Zizyphus mauritiana* against *Pseudomonas fluorescens*

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ABSTRACT

The use of medicinal and aromatic plants to treat diseases is vital in the safety of fish communities. The *Ziziphus mauritiana* show appearance the phenolic compounds that proved had an antimicrobial effect. This study aimed to investigate the phytochemicals composition of *Zizyphus mauritiana* and its antimicrobial activity against *P. fluorescens*. *Ziziphus mauritiana* extraction by maceration methods using methanol solvent. The phytochemical screening was obtained for Alkaloids, Saponins, Triterpenoids, Steroids, and Tannins. The bacterial strain was purchased from Fish Parasites and Diseases, Faculty of Fisheries and Marine Sciences of University of Brawijaya, Malang. Determination of Antibacterial activity using the disc diffusion methods. This study showed the presence of some secondary metabolites such as Alkaloids, Saponins, Triterpenoids, Steroids, Lignin, Phenol, and Tannins. Leaves extract of *Ziziphus mauritiana* also showed antimicrobial activity against *Pseudomonas fluorescens* at different concentrations (25, 50, 100, 250, 500 µg/µl and control using antibiotics oxytetracycline of 31,2 µg/µl). The conclusion of this study was the maximum zone of inhibition showed at a concentration of 500 ppm. Antimicrobial activity of *Ziziphus mauritiana* leaves extract caused by the presence of tannins and alkaloids compounds.

Key words : *Ziziphus mauritiana, Extract, Phytochemical, Antibacterial, Pseudomonas fluorescens.*

Introduction

Pseudomonas fluorescens is a Gram-negative bacterium existing widely in soil, water, plant, and animals. *They are* a common bacterial pathogen to a wide range of aquaculture animals, including various fish species. In aquaculture, it is a common pathogen for shrimp and a wide range of fish species (Swain *et al.,* 2007). In addition, *P. fluorescens* can also infect humans and is known to cause outbreaks of bacteremia (Gershman *et al.,* 2008). *Pseudomonas fluorescens* is an aquaculture pathogen that can infect many fish species, including Indian major carps, black carp, common carp, and Japanese flounder (Geng *et al.,* 2006; Swain *et al.,* 2007). Infection of fish by *P. fluorescens* leads to the development of the so-called Red Skin Disease, which can occur all year round and especiallyin fish injured by, for example, inappropriate handling and transportation. Owing to the lack of adequate means ofcontrol, the disease often leads to mortality, thus causing heavy economic losses. Studies on the pathogenesis of aquaculture-associated *P. fluorescens* are scarce, and this bacterium's virulence mechanisms of this bacterium remainlargely obscure.

The use of medicinal plants for the treatment of

diseases is widely explored and used (Islamy *et al.,* 2017; Kilawati and Islamy, 2019) and the use of extracts from animal secondary metabolites (Islamy, 2019). Medicinal plants receive attention to research centers because of their particular importance in the safety of communities (Najafi *et al.,* 2013). Among all the family genus, Rhamnaceae members of the *Ziziphus* have been used for centuries in folk medicine. About 40 species of *Ziziphus* are available, one of which is *Ziziphus mauritania* Lam., very common. Carbohydrates, starch, proteins, sugar, mucilage, and vitamins are abundantly present in *Ziziphus* species; *Ziziphus mauritana* is generally grown in dry places (Parmar *et al.,* 2012). The methanolic extract of *Ziziphus mauritana* indicated that the plant exhibited antimicrobial activity. The secondary metabolites identified in the *Ziziphus mauritiana* showthe appearance of the phenolic compounds with antimicrobial effects (Najafi, 2013). Based on the information above, this study aimed to investigate the phytochemicals composition of *Zizyphus mauritiana* and its antimicrobial activity against *P. fluorescens*.

Materials and Methods

Leave extraction

Ziziphus mauritiana old leaves collected from west Wendit village, Pakis, Malang on dry season. Extraction of *Z. mauritiana* based on research conducted by Virgianti and Purwati (2015), the leaves were collected and cleaned using water and shade dried for 4 days. Dry leave, then ground to get powder. 50 g of *Z. mauritiana* powder was macerated with 500 mL of methanol solvent for 48 hours (1/10 w / v) base on the published method (Sumiati, 2014). after 48 hours, the aqueous extract is filtered using Whatman No. 1 filter paper then evaporated using a rotary evaporator at 40 °C. The methanol extract was then placed into a glass bottle.

Phytochemical screening

Phytochemical screening obtained for the presence of Alkaloids, Saponins, Triterpenoids, Steroids, Lignin, Phenol and Tannins using standard experimental procedure (SS handa *et al.,* 2008).

Bacterial strains and preparation

The bacterial strain was purchased from the laboratory of Fish Parasites and Diseases, Faculty of Fisheries and Marine Sciences of University of Brawijaya, Malang. Three to five colonies were suspended in 5 ml of TSB and incubated overnight without shaking.

Determination of Antibacterial activity

This step was obtained using the disc diffusion method (Kirby *et al.,* 1986). Muller Hinton so that plateswere inoculated with 0.2 ml each bacterium suspension overnight culture is adjusted to a 0.5 McFarland turbidity standard (10^8 colony-forming units [CUF/ml]) and uniformly spread out. The plates were incubated at 37 °C for 24 hr (Onkar *et al.,* 1995). After the incubation period, the inhibition zone was around the discs, which was measured and recorded.

Results and Discussion

The phytochemical analysis was obtained to confirm Alkaloids, Saponins, Triterpenoids, Steroids, Lignin, Phenol, and Tannins. The details of phytochemical screening are given in Table 1. showed the presence of some secondary metabolites such as Alkaloid, Triterpenoid, Steroid, and Tanin.

The antimicrobial assay of the methanolic extract of *Ziziphus mauritana* indicated that the plant has antimicrobial activity potentials against the *P. fluorescens* at three different concentrations of 25, 50, 100, 250, 500 µg/µl and control using antibiotics oxytetracycline of 31,2 µg/µl; and the zone of inhibition was recorded and showed in Table 2.

In the present investigation, leaves extract of *Ziziphus mauritiana* showed antimicrobial activity against *Pseudomonas fluorescens* at different concentrations. The inhibition zone increased simultaneously with increasing concentration of *Ziziphus mauritiana* leaves extract. The maximum zone of inhibition showed at a concentration of 500 ppm. We assume that the antimicrobial activity of *Ziziphus mauritiana* leaves extract is caused by the presence of tannins and alkaloids compounds.

Tannins are present in many plants and have various physiological effects like antimicrobial and antiparasitic (Islamy *et al.,* 2017). Tannins have general antimicrobial and have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al.,* 1991). The growth of many fungi,yeasts, bacteria, and viruses was inhibited by tannins (Chung *et al.,* 1998).

Alkaloids are secondary plant metabolites with

Table 1. Result of Phytochemical screening of Z. mauritiana leaves extract

Test	Result	Indicator
Flavonoids	+	Formed pink
Alkaloid (dragendroff)	+	No white precipitate formed
Alkaloid (mayer)	++	No orange precipitate formed
Lignin	+	Brownish-yellow
Saponin	-	Absence of permanent foam
Triterpenoid	+	Absence of permanent foam
Phenol	-	No blackish-green colour
Steroid	+	the color turns green
Tanin	++	the color turns greenish black

Table 2. The results of the antimicrobial assay of the methanolic extract of *Ziziphus mauritana* against *Pseudomonas fluorescens* with 3 repetition

Concentration (µg/µl)	Zone of inhibition (mm)			Average	Std±
	1	2	3		
25	4.3	5.2	5.3	4.93	±0.55
50	6.0	5.9	5.7	5.86	±0.15
100	6.6	6.9	7.1	6.86	±0.25
250	8.1	7.8	8.3	8.06	±0.25
500	9.5	10.5	10.5	10.16	±0.58
Control	10.9	11.6	11.1	11.2	±0.36

potent pharmacological activitiesas antibacterial properties (Li *et al.,* 2019). alkaloid extract also showed significant antibacterial activity second to *C. citrinus*. Similar margins of inhibition were obtained by Aliyu *et al.* (2011). A bacteriostatic effect against *P. aeruginosa* by all plant alkaloid extracts could be due to the pathogen's thick outer membrane that is highly hydrophobic and possibly provided a permeability barrier to the extract (Chitemerere and Mukanganyama, 2011).

This preliminary research needs to improve and develop. Further research should test this extract using an animal test. A Study shows that *Ziziphus mauritania* can improve the immune response of tilapia (El Asely *et al.,* 2020), wherein that invasive non-native fish (Insani *et al.,* 2020; Hasan and Tamam, 2019) has been widely cultivated as animal protein source community. Moreover, we recommend using native fish as test animals, such as Lobocheilos fish (Hasan *et al.,* 2019) and Beardless barb (Hasan *et al.,* 2021). Other fish can be used as test animals if they have high economic value for both consumption and ornamental fish, such as Giant snakehead fish (Pratama *et al.,* 2019), and alligator gar (Hasan *et al.,* 2020) even mangrove gastropods (Islamy and Hasan, 2020). In addition, these fish need to meet the criteria for a good test animal (Islamy and Hasan, 2021).

Conclusions

This study concludedthat the maximum zone of inhibition showed at a concentration of 1000 µg/µl. Antimicrobial activity of *Ziziphus mauritiana* leaves extract caused by the presence of tannins and alkaloids compounds.

References

Aliyu, A.B., Musa, A.M., Abdullahi, M.S., Ibrahim, H. and Oyewale, A.O. 2011. Phytochemical screening and antibacterial activities of Vernoniaambigua, Vernoniablumeoides and Vernoniaoocephala (asteraceae). *Acta Poloniae Pharmaceutica—Drug Research*. 68(1) : 67-73.

Bauer, A.W., Kirby, M.M., Sherris, J.C. and Truck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 45 : 493-496.

Chitemerere, T. A. and Mukanganyama, S. 2011. *In-vitro* antibacterial activity of selected medicinal plants from Zimbabwe. *African Journal of Plant Science and Biotechnology*. 2011; 5 : 1–7.

Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W. and Lin, Y. 1998. Tannins and human healt: a review. *Crit Rev Food Sci Nutr*. 38 (6): 421-464.

El Asely, A., Amin, A., Abd El-Naby, A. S., Samir, F., El-Ashram, A. and Dawood, M. A. O. 2020. *Ziziphus mauritiana* supplementation of Nile tilapia (Oreochromisniloticus) diet for improvement of immune response to Aeromonashydrophila infection.

Fish Physiology and Biochemistry. 46(4) : 1561–1575. https://doi.org/10.1007/s10695-020-00812-w

Geng, X., Ding, S., Sun, H. and Xie, J. 2006. Immunogenicity and protective effects of *Pseudomonas fluorescens* vaccines on grass carp. *J. Southwest Agric. Univ. (Natural Science)* 28 : 120–123 (in Chinese, with English abstract)

Gershman, M.D., Kennedy, D.J., Noble-Wang, J., Kim, C., Gullion, J. and Kacica, M. 2008. Multistate outbreak of *Pseudomonas uorescens* bloodstream infection after exposure to contaminated heparinized saline ush prepared by a compounding pharmacy. *Clin. Infect. Dis.* 47 : 1372–1379. doi: 10.1086/592968

Handa, S.S. 2008. An overview of extraction techniques for medicinal and aromatic plants. In: Handa et al. (eds), *Extraction Technologies for Medicinal and Aromatic Plants.* (International Centre for Science and High Technology. Trieste. Italy. 21-54.

Hasan, V., Soemarno, Widodo, M.S., Wiadnya, D.G.R., Mukti, A.T. and Irawan, B. 2019. Distribution extension and first record of *Lobocheilos falcifer* (Cypriniformes, Cyprinidae) in Central Java Province, Indonesia. *Ecology, Environment and Conservation*. 25 (July Suppl. Issue) : S158-S161.

Hasan, V., Wijayanti, A., Tamam, M. B., Islamy, R. A. and Widodo, M. S. 2021, February). Beardless barb Cyclocheilichthys apogon (Valenciennes, 1842)(Cypriniformes, Cyprinidae): Distribution extension and first record from South Bali. In: *IOP Conference Series. Earth and Environmental Science* (Vol. 679, No. 1). IOP Publishing.

Hasan, V. and Tamam, M.B. 2019. First record of the invasive Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758) (Perciformes, Cichlidae),on Bawean Island, Indonesia. *Check List.* 15 (1) : 225-227. DOI: 10.15560/15.1.225

Hasan, V., Widodo, M.S., Islamy, R.A., Pebriani, D.A.A. 2020a. New records of alligator gar, *Atractosteus spatula* (Actinopterygii: Lepisosteiformes : Lepisosteidae) from Bali and Java, Indonesia. *Acta Ichthyologica et Piscatoria* 50 (2): 233-236.

Insani, L., Hasan, V., Valen, F.S., Pratama, F.S., Widodo, M.S., Faqih, A.R., Islamy, R.A., Mukti, A.T. and Isroni, W. 2020. Presence of the invasive nile tilapia *Oreochromis niloticus* Linnaeus, 1758 (perciformes, cichlidae) in the yamdena island, indonesia. *Ecology, Environment and Conservation*. 26 (3) : 1115-1118.

Islamy, R.A. and Hasan, V. 2020. Checklist of mangrove snails (Mollusca: Gastropoda) in South coast of Pamekasan, Madurai Island, East Java, Indonesia. *Biodiversitas.* 21 : 3127-3134.

Islamy, R. A. 2019. Antibacterial Activity of Cuttlefish Sepia sp. (Cephalopoda,) Ink extract against *Aeromonas hydrophila*. *Majalah Obat Tradisional*. 24(3): 184.

Islamy, R. A. and Hasan, V. 2021. Pestisida terhadap ekosistem, ikan dan organisme akuatik. Purwokerto, Central Java: CV Pena Persada.

Kilawati, Y. and Islamy, R. A. 2019. The Antigenotoxic Activity of Brown Seaweed (*Sargassum* sp.) Extract Against Total Erythrocyte and Micronuclei of Tilapia *Oreochromis niloticus* Exposed by Methomyl-Base Pesticide. *The Journal of Experimental Life Sciences.* 9(3) : 205–210.

Li, Q., Zhao, Y. L., Long, C. B., Zhu, P. F., Liu, Y. P. and Luo, X. D. 2019. Seven new veratramine-type alkaloids with potent analgesic effect from Veratrum taliense. *Journal of Ethnopharmacology*. 244 : 112137. https://doi.org/10.1016/j.jep.2019.112137

Mabhiza, D., Tariro, C. and Stanley, M. 2016. Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernoniaadoensis against Staphylococcus aureus and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*. 2016, Article ID 6304163, 7 pages, 2016. https://doi.org/10.1155/2016/6304163.

Najafi, S. 2013. Phytochemical screening and antibacterial activity of leaf extract of *Ziziphus mauritiana* Lam. *Intl. Res. J. Appl. Basic. Sci.* 4 : 3274-3276.

Onkar, D., Dhigra. and James, B. 1995. Basic plant pathology methods. 287-305.

Parmar, P., Bhatt, S., Dhyani, S. and Jain, A. 2012. Phytochemical studies of the secondary metabolites of *Ziziphus mauritiana* Lam. Leaves. *International Journal of Current Pharmaceutical Research.* 4(3) : 153-155.

Pratama, W. W., Nursyam, H., Hariati, A. M., Islamy, R. A. and Hasan, V. 2020. Short Communication: Proximateanalysis, aminoacid profile and albumin concentration of variousweights of Giant Snakehead (Channamicropeltes) from Kapuas Hulu, West Kalimantan, Indonesia. *Biodiversitas Journal of Biological Diversity*. 21(3).

Purwati, D.M. and Virgianti, D.P. 2015. Daya Hambat Ekstrak Etanol Daun Binahong (*Anrederacordifolia* (Ten.) Steenis) Terhadap Pertumbuhan Bakteri *Streptococcus pyogenes* SecaraIn Vitro. *Jurnal Kesehatan Bakti Tunas Husada.* 13(1) : 24-27.

Sodipo, O.A., Akanji, M.A., Kolawole, F.B. and Odutuga, A.A. 1991. Saponin in the active antifungal principle in Garcinia kola, heckle seed. *Biosci. Res. Commun.* 3: 171.

Sumiati, E. 2014. UjiAktivitas Antibakteri Ekstrak Kloroformdan Ekstrak Etanol Biji Bidara Laut (*Strychnosligustrina* Bl) Terhadap *Staphylococcus aureus* ATCC 25923 and *Salmonella thypi. Jurnal Ilmiah Biologi.* 2(1) : 1-10.

Swain, P., Behura, A., Dash, S. and Nayak, S.K. 2007. Serum antibody response of Indian major carp, *Labeo rohita* to three species of pathogenic bacteria; *Aeromonas hydrophila*, Edwardsiellatarda and Pseudomonas uorescens. *Vet. Immunol. Immunopathol.* 117 : 137–141. doi: 10.1016/j.vetimm.2007.02.010