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Phytofungitoxic Agent from Wild Plants

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Abstract

The objective of research was to find out phytofungitoxic agent from a methanol extract of *Barringtona racemosa* Linn., *Datura metel* Linn., *Euphorbia hirta* Linn., and *Hydnophytum formicarum* Jack. on *Saprolegnia* sp. infected catfish eggs. The a one-way ANOVA test result showed that the methanol extracts of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum* gave the significant effect (p = 0.05) to mortality of *Saprolegnia* sp. Hatching rate (mean ± SE) of *Saprolegnia* sp. infected catfish eggs immersed the extract of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum* was 78.8 ± 2.7, 93.5 ± 0.6, 92.8 ± 1.7, and 79.8 ± 1.8%, respectively. It shown that the treatment of 5, 20, 30, and 40 ppm was a concentration of *B. racemosa*, *D. metel*, *E. hirta* and *H. formicarum*, respectively resulted the highest hatching rate of the infected catfish eggs. The study stated that the extracts of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum* have antifungal property to *Saprolegnia* sp. The extracts can be used as a natural antifungal agent for controlling saprolegniasis.

Keywords: Antifungus, *Saprolegnia* sp., *Barringtonia racemosa, Ephorbia hirta, Datura metel, Hydnophytum formicarum*, Hatching rate

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1. Introduction

Fungi are heterotrophic organisms that are unable to produce their own food through photosynthesis due to lack of chlorophyll. Therefore, saprophytic fungi use resource living on dead organic matter dissolved, destroying the remains of dead plants or animals, or make complex compounds into simpler compounds as food [1]. One form of parasitism in ponds is saprolegnia caused by the fungus *Saprolegnia* sp. The fungus can attack all kinds of fish in all kinds of environments [2] due to the fungus can live in water with a range temperature of 3-33 °C [3]. The visible sign of *Saprolegnia* sp. attacked on fish and eggs is an organ or an egg covered by fungal mycelium looked like cotton. The fish eggs attacked this fungus will not hatch and this fungus will blowout infection [4]. The use of plant based fungicide should be encouraged because it disintegrates easily into constituent elements without leaving any permanent product in different regions of the environment. The present research work was to find out the antifungus *Saprolegnia* sp. from existing plants and abundant around the fish farming location of Aceh Besar District, Aceh Province - Indonesia. These natural antifungals are ecofriendly and require only a short time to prepare them at a low cost. A number of wild plants around fish farming area have screened their potential antifungus to *Saprolegnia* sp. Based on preliminary tests, the wild plant extracts that showed inhibition of the fungal growth above 75% at a concentration of 50 ppm used as an antifungal candidate. Concentration of fungicide in aquatic system should be minimized so there is no disadvantageous effect on the surrounding. These four wild plants shown in figure 1 were chosen because they encountered the criteria.

Plant *Barringtonia racemosa* L. (Lecythidaceae) can be found in tropical rain forest and in the saline areas of mangrove swamps [5]. Methanol extracts of the seeds contain saponins and flavonoids [6]. The methanolic seed extract show toxicity [6], molluscicidal [7,8], cercariacidal, mosquito larvacidal, antiplasmodial [8], anti hatching [9], immunomodulatory [10], anti-inflammatory, analgesic [11], , antioxidant [12], antitumor [13], and antibacterial [14] properties. Extracts of the fruit are used against malaria, cough, asthma, jaundice, headache, eye inflammation, diarrhoea and sores [15].

Figure 1: The four wild plants used in this study

Datura metel L. (Solanaceae) is a wild plant that grows well in sandy soil, shrubs, open grassland, or riverside. All parts of the plant contains alkaloids, saponins, and flavonoids [16]. The leaves have anaesthetic, anodyne, anti-asthmatic, antispasmodic, antitussive, bronchodilator, hallucinogenic, hypnotic and mydriatic [17] properties. It has a wide range of applications including in the treatment of epilepsy, hysteria, insanity, heart diseases, fever with catarrh, diarrhoea, skin diseases [18].

Euphorbia hirta L. (Euphorbiaceae) is a plant that grows well in a meadow on the banks of the river. The leaf extract has an active compound in the form of tannins, flavonoids, terpenoids, alkaloids, and polyphenolic compounds [19]. The methanol extract showed antifungal activity by examining the barriers fungus *C. capsici*, *F. pallidoroseum, B. theobromae, A. alternate, P. citrinum, P. carica papaya,* and *A. nigar* [20].

Hydnophytum formicarum Jack (Rubiaceae) is a genus of epiphytic myrmecophytes grown in tree branches and on trunks, and known as ant plants or ant-house plants [21]. The inhabitant use it as medication to treat swelling, headaches and rheumatism, cholera, and cancer [22]. Phytochemical analysis showed the plant contains flavonoids and tannins that have anticancer [23,24], antimicrobial, antioxidative [25,26,27], cytotoxic [28], and antiproliferative [29] properties.

2. Materials and Methods

2.1. Collection of plant material

The plant material was collected from Krueng Cut (5° 35’ 34.53” N 95° 20’ 57.45” E) and Lhok Seudu (5° 20’ 14” N 95° 14’ 28” E) villages of Aceh Besar District, in January 2014-January 2015, authenticated by Botanist of Syiah Kuala University.

2.2. Preparation of plant sample

The aerial parts of the plant were air dried under shade for eight days. The amount of moisture removed was calculated and the sample was then stored in a herb room at 10 °C with a relative humidity of less than 50% until used.

2.3. Extraction of plant substituents

The dried plant material was ground in a mechanical grinder, sieved through 40 meshes, extracted with 96% (v/v) methanol for 24 hours, concentrated, dried under vacuum at 65 °C and used crude extract for experiments. The previous studies reported the phytochemical screening of the methanol extract of the plants as shown in table 1.

2.4. Bioassay tests

The acute toxicity experiments were conducted in the Laboratory of Officially Technical Implementation Unit for Freshwater Aquaculture (UPTD-BAT) Jantho, Aceh Besar District in four replications for each experimental unit in a completely randomized design using extracts of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum*

against fungus *Saprolegnia* sp. Each extract was dissolved in water to make concentration 5 to 50 ppm on the basis of preliminary testing. A hundred of *Saprolegnia* sp. infected catfish eggs was immersed into a vessel (0.5 L) containing 300 mL of treated extract for 15 minutes. Hatchability was recorded 24 hours later. The main parameter observed in this study was hatching rate of *Saprolegnia* sp. infected catfish eggs immersed the plant extracts. The physic-chemical water parameters, i. e. pH, temperature, dissolved oxygen were also observed.

Table 1: Phytochemical screening of the plants

Constituents	*B. racemosa*[6]	*D. metel*[16]	*E. hirta*[19]	*H. farmicarum*[26]
Terpenoids	-	-	+	-
Steroids	+	-	-	+
Alkaloids	-	+	+	-
Saponin	+	+	-	-
Flavonoids	+	+	+	+
Tannins	-	-	+	+

2.5. Statistical analysis

Data analyses were performed with SPSS version 18.0. The results were analyzed for each experimental unit separately using a one-way ANOVA followed by Duncan's test at 5% of significance. For each experimental unit, the four replicates used for each extracts concentration yielded a hatching rate percentage.

3. Results and Discussion

Data percentage hatchability of *Saprolegnia* sp. infected catfish eggs was showed in table 2. The a one-way ANOVA results showed that there were a significant effect of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum* extracts on inhibition of *Saprolegnia* sp. at the p =0.05 [F(6,21) =21.9, p_{sig} =0.0], [F(6,21) =501.9, p_{sig} =0.0], [F(6,21) =58.1, p_{sig} =0.0], and [F(6,21) =134.8, p_{sig} =0.0], respectively.

The observations on control showed that the *Saprolegnia* sp. infected eggs were covered by mycelium resembled fine threads like cotton. According to [30], the infected egg cannot develop properly into the embryo as fungal hyphae absorb glukoprotein of the egg as a nutrient, then the egg will not hatch. The highest percentage of eggs hatchability was found in treatments of *B. racemosa, D. metel, E. hirta, and H. formicarum* extracts at concentration of 5, 20, 30, and 40 ppm in which the eggs have been able to hatch as much as 78.8 ± 2.7, 93.5 ± 0.6, 92.8 ± 1.7, and 79.8 ± 1.8%, respectively. It was explained that the extracts contained saponins, flavonoids, and tannins enforced and delayed the development of mycelium *Saprolegnia* sp. on the egg chorion. The previous studies stated that saponins and flavonoids are often use for antifungal compounds that work damaging the cell walls and cytoplasmic membrane that can inhibit the growth of fungus [31,32,33,34]. Meanwhile, tannins can kill fungal growth by means of the protein precipitate and cause fungal cell membrane shrinks resulting changes in cell permeability [35,36].

Table 2: Percent hatchability of *Saprolegnia* sp. infected fish eggs immersed to the methanol extracts of plants

Conc. extract (ppm)	% Hatchability (mean ± SE*, n = 400)			
	B. racemosa	*D. metel*	*E. hirta*	*H. formicarum*
Control	46.0 ± 3.8[b]	31.3 ± 1.5[a]	41.8 ± 3.5[a]	25.0 ± 1.1[a]
5	78.8 ± 2.7[c]	29.8 ± 1.4[a]	47.8 ± 2.6[a]	43.8 ± 1.4[b]
10	71.8 ± 4.5[c]	32.8 ± 1.1[a]	49.0 ± 0.4[a]	54.8 ± 0.9[c]
20	75.3 ± 3.1[c]	93.5 ± 0.6[d]	70.0 ± 2.7[b]	59.5 ± 0.6[d]
30	54.0 ± 2.5[b]	64.5 ± 0.5[c]	92.8 ± 1.7[c]	67.3 ± 2.4[e]
40	54.0 ± 3.6[b]	38.5 ± 0.9[b]	70.3 ± 0.9[b]	79.8 ± 1.8[f]
50	32.3 ± 4.8[a]	37.5 ± 0.9[b]	73.5 ± 3.3[b]	55.0 ± 1.5[c]

*Means followed by the same letter are not significantly different at 5% level (Duncan's test following ANOVA).

The hatchability of eggs results revealed inversely proportion to the higher concentration of the given extracts caused the hatchability declined. This is caused by the effect of saponins in the extracts that able to make eggs failed to hatching. Saponins are natural surfactants, act as powerful emulsifiers that have hemolytic ability, which may in turn disrupt the integrity of the chorion and hence increase the permeability to chemicals may affect any lipid structure located on the outer membrane of the chorion. Saponins at higher concentrations caused shrinkage of the chorion, which led to the failure of hatching [37].

Data physico-chemical parameters of water were measured at the beginning and the end of treatment. Observation of physico-chemical quality of the water indicated that the value of the range of all parameters remained within the tolerance limits of hatching eggs. Data obtained based on observations at the time of hatching eggs temperatures ranging from 25-26.4 °C, pH range 6-8 and dissolved oxygen ranged from 6.4- 11.8 ppm. These values support the incubation and hatching of eggs in accordance with the criteria of the range of values of temperature, pH, and dissolved oxygen normally.

4. Conclusion

Gather together the clues, the methanol extracts of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum* showed potent antifungal activity against *Saprolegnia* sp. fungus. The findings of the present study suggest the use of extracts of *B. racemosa*, *D. metel*, *E. hirta*, and *H. formicarum* in the development of agents for the inhibition of saprolegniasis infection.

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References

[1] G.A. Neish, and G.C. Hughes. *Diseases of fishes*, Book 6, Fungal Diseases of Fishes. T.W.F. Publications, Neptune, New Jersey, 159. 1980.

[2] F.P. Meyer. "Aquaculture disease and health management". *J. Anim. Sci*., 69: 4201-4208. 1991.

[3] S. Aly, and A. El-Ashram. "Some factors contributing to the development of saprolegniasis in nile tilapia (*Oreochromis niloticus*)". *Alexandria Journal Veterinary Sciences*, 16: 165-174. 2000.

[4] G.E Howe, W.H. Gingerich, V.K. Dawson, and J.J. Olson. "Efficacy of hydrogen peroxide for treating Saprolegniasis in channel catfish". *J. Aquat. Anim. Health*, 11: 222-230.1999.

[5] P. Chantaranothai. "*Barringtonia* (Lecythidaceae) in Thailand". *Kew Bulletin*, 50: 677-694. 1995.

[6] M. Musman. "Toxicity of *Barringtonia racemosa* (L.) Kernel Extract on *Pomacea canaliculata* (Ampullaridae)". *Tropical Life Sciences Research*, 21: 41-50. 2010.

[7] M. Musman, S. Karina, and F. Rizki. "Saponins Extract from *Barringtonia racemosa* as Molluscicide to Brackishwater Pond Snails (*Cerithidea cingulata*)". *International Journal of Applied Research and Technology*, 3: 92-97. 2014.

[8] J.A.O. Ojewole, N. Nundkumar, and C.O. Adewunmi. "Molluscicidal, Cercariacidal, Larvicidal and Antiplasmodial Properties of *B. racemosa* Fruit and Seed Extracts". *BLACPMA*, 3: 88-92. 2005.

[9] M. Musman, S. Kamaruzzaman, S. Karina, R. Rizqi, and F. Arisca. "A preliminary study on the anti hatching of freshwater golden apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) eggs from *Barringtonia racemosa* (Magnoliopsida: Lecythidaceae) seeds extract". *AACL Bioflux*, 6: 394-398. 2013.

[10] P.R. Patil, M.R. Patil, A. Mane, and S. Patil. "Immunomodulatory effects of fruits of *Barringtonia racemosa* Linn". *Int J Basic Clin Pharmacol*., 2: 216-219. 2013.

[11] P. Shikha, P.G. Latha, S.R. Suja, G.I. Anuja, S. Shyamal, V.J. Shine, S. Sini, N.M. Krishnakumar, and S. Rajasekharan. "Anti-inflammatory and Analgesic Activity of *B. racemosa* Roxb. Fruits". *Indian Journal of Natural Products and Resourced*, 1: 356-361. 2010.

[12] M. H. Nurul, M. Radzali, R. Johari, A. Syahida, and M. Maziah. "Antioxidant Activities of Different Aerial Parts of Putat (*Barringtonia racemosa* L)". *Malaysian Journal of Biochemistry and Molecular Biology*, 16: 15-19. 2008.

[13] T. J. Thomas, B. Panikkar, A. Subramoniam, M. Nair, and K.R. Penikkar. "Short communication,. Antitumor property and toxicity of *Barringtonia racemosa* Roxb seed extract in mice". *J Ethnopharmacol*., 82: 223-227. 2002.

[14] S. Khan, A. Jabbar, C.M. Hasan, and M.A. Rashid. "Antibacterial activity of *Barringtonia racemosa*". *Fitoterapia*, 72: 162-164. 2001.

[15] M. Behbahani, A.M. Ali, R. Muse, and N.B. Mohd. "Antioxidant and anti-inflammatory activities of leaves of *Barringtonia racemosa*". *Journal of Medicinal Plants Research*, 7: 95-102. 2007.

[16] H. I. Kankia. "Phytochemical analysis and antimicrobial activity of crude extract of *Datura metel* leaves". *Arch. Appl. Sci. Res*., 6: 138-143. 2014.

[17] H. Zhong, Y.Y. Yan, J. Suo, and G.W. Hui. *A Barefoot Doctors Manual: The American Translation of the Official Chinese Paramedical Manual*, Running Press, Philadelphia, 942. 1977.

[18] K. A. Kiruthika, and R. Sornaraj. "Screening of bioactive components of the flower *Datura metel* using the GC-MS technology". *Int. Pharm Tech Res.*, 3: 2025-2028. 2011.

[19] M. Qaisar, S.N. Gilani, S. Farooq, A. Rauf, R. Naz, Shaista, and S. Perveez. "Preliminary Comparative Phytochemical Screening of *Euphorbia* Species". *Am-Euras. J. Agric. & Environ. Sci.*, 12: 1056-1060. 2012.

[20] Y. Pounikar, P. Jain, N. Khurana, S. Patil, L.K. Omray, and A. Gajbhiye. "Medicinal important of *Euphorbia hirta* Linn". *Scholar Academic Journal of Pharmacy*, 2: 241-246. 2013.

[21] R. W. Elliot, D.L. Jones, and T. Blake. *Encyclopaedia of Australian Plants Suitable for Cultivation*, vol. 5. Port Melbourne: Lothian Press, 392–93. 1990.

[22] C. R. Huxley. "The ant-plants Myrmecodia and Hydnophytum (Rubiaceae), and the relationships between their morphology, ant occupants, physiology and ecology". *New Phytologist*, 80: 231–268. 1978.

[23] T. Senawong, S. Misuna, S. Khaopha, S. Nuchadomrong, P. Sawatsitang, C. Phaosiri, A. Surapaitoon, and B. Sripa. "Histone deacetylase (HDAC) inhibitory and antiproliferative activities of phenolic-rich extracts derived from the rhizome of *Hydnophytum formicarum* Jack.: sinapinic acid acts as HDAC inhibitor". *BMC Complementary and Alternative Medicine*, 13: 232. 2013.

[24] H. Abdullah, A.H.L. Pihie, J. Hohmann and J. Molnar. "A natural compound from *Hydnophytum formicarium* induces apoptosis of MCF-7 cells via up-regulation of Bax". *Cancer Cell International*, 10: 14. 2010.

[25] S. Prachayasittikul, R. Pingaew, V. Yamkamon, A. Worachartcheewan, S. Wanwimolruk, S. Ruchirawat, and V. Prachayasittikul. "Chemical Constituents and Antioxidant Activity of *Hydnophytum formicarum* Jack". *International Journal of Pharmacology*, 8: 440-444. 2012.

[26] R. Ahmad, E.N.M. Mahbob, Z.M. Noor, N.H. Ismail, N.H. Lajis, and K. Shaari. "Evaluation of antioxidant potential of medicinal plants from Malaysian Rubiaceae (subfamily Rubioideae)". *African Journal of Biotechnology*, 9: 7948-7954. 2010.

[27] S. Prachayasittikul, P. Buraparuangsang, A. Worachartcheewan, C.I.N. Ayudhya, S. Ruchirawat, and V. Prachayasittikul. "Antimicrobial and Antioxidative Activities of Bioactive Constituents from *Hydnophytum formicarum* Jack". *Molecules*, 13: 904-921. 2008.

[28] A. Itharat, and B. Ooraikul. "Research on Thai medicinal plants for cancer treatment". *Advances in Medicinal Plant Research*, 13: 287-317. 2007.

[29] J. Y. Ueda, Y. Tezuka, A.H. Banskota, Q. Le Tran, Q.K. Tran, Y. Harimaya, I. Saiki, and S. Kadota. "Antiproliferative activity of Vietnamese medicinal plants". *Biological and Pharmaceutical Bulletin*, 25: 753-760. 2002.

[30] M. N. Lingga, I. Rustikawati, and I.D. Buwono. "Effectiveness flower extract of *Nicolaila speciosa* Horan for the prevention of *Saprolegnia* sp. on sangkuriang catfish eggs". *Journal of Fisheries and Marine*, 3: 75-80. 2012 [Indonesia]

[31] Y. Matsumoto, K. Kaihatsu, K. Nishino, M. Ogawa, N. Kato, and A. Yamaguchi. "Antibacterial and Antifungal Activities of New Acylated Derivatives of Epigallocatechin Gallate". *Front Microbiol*., 3: 53. 2012.

[32] A. Kandalkar. "Free radical scavenging activity of *Euphorbia hirta* Linn. Leaves and isolation of active flavonoid myricitrin". *Asian Journal of Pharmaceutical and Clinical Research*, 3: 234-237. 2010.

[33] T. P. T. Cushnie, and A. J. Lamb. "Antimicrobial activity of flavonoids". *Int J Antimicrob. Agents*, 26: 343-356. 2005.

[34] K. O. Soetan, M.A. Oyekunle, O.O. Alyelaagbe, and M.A. Fafunso. "Evaluation of the antimicrobial activity of saponins extract of Sorghum bicolor L Moench". *Afr. J. Biotechnol.*, 5: 2405-2407. 2006.

[35] R. I. Okoli, A.A. Turay, J.K. Mensah, and A.O. Aigbe. "Phytochemical and antimicrobial properties of four herbs from edo state, Nigeria". *Report and Opinion*, 1: 67-73. 2009.

[36] N. M. Hussin, R. Muse, S. Ahmad, J. Ramli, M. Mahmood, M.R. Sulaiman, M.Y.A. Shukor, M.F.A. Rahman, and K.N.K. Aziz. "Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae)". *African Journal of Biotechnology*, 8: 2838-2842. 2009.

[37] M. K. Ahmad, and B. A. Ansari. "Toxicity of Neem Based Pesticide Azacel to the Embryo and Fingerlings of Zebrafish *Danio rerio* (Cyprinidae)". *World J. Zool*., 6: 47-51. 2011.