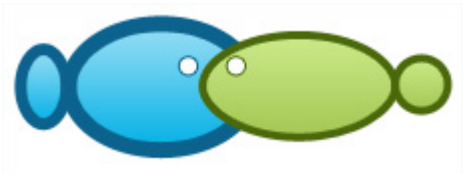

The effect of tannin from red betel (*Piper crocatum*) leaves towards blood biochemistry and histology of North African catfish (*Clarias gariepinus*)

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Abstract. The consequences of blood biochemistry changes in fish which was exposed to tannin compound (poliphenol) have not been investigated. Furthermore, the impact of plant decomposition in certain water is poorly understood. North African catfish (*Clarias gariepinus*) was injected with a single dose of *Piper crocatum* tannin. The fish was exposed (LD 25, 50 and 75% from 96 hours LC50) to tannin concentration with the dose of 0.36, 1.08 and 1.80 mg/kg, and (75% of 96-hour LC50) to phenol concentration (3 mg/kg) and *P. crocatum* tannin (1.8 mg/kg). The effects of tannin on hemoglobin and blood biochemistry were measured 3 days after injection. The erythrocyte on hemoglobin and hematocrit decreased significantly. The blood biochemistry such ALP (alkaline phospatase) and glucose also significantly decreased. Meanwhile, there were some increase in blood urea nitrogen (BUN) and cortisol hormone. The histopathological analysis of fish kidney and liver after phenolic exposure indicated some tissue damage such as hypertrophy, congestion, and necrosis. This is the first report on effect of *P. crocatum* leaves tannin on blood biochemistry and histopathology.
Key Words: poliphenol, *P. crocatum*, blood biochemistry, hemoglobin, erythrocyte, hematocrit.

Introduction. Traditional medicines derived from medicinal plants are used by about 60% of the world's population (Firdaus et al 2015; Chamidah et al 2017). In Indonesia, many plant exhibit bioactive compounds which has potency to be used for therapy such antibacterial, hypo-cholesterol, atherosclerosis, hypertension (Harjana 2011; Prihanto et al 2012). *Piper crocatum* leaves is one of the plant that has enormous therapy applications. Dewi et al (2014) states that *P. crocatum* leafs can lower blood sugar and cholesterol levels in Wistar rats' trial. Harjana (2011) also confimed the *P. crocatum* leaf capability on decreasing cholesterol level.

In nature, decomposition of plant materials more or less contributes to phenol accumulation in a water environment (Ali et al 2011). This compound may affect the life of aquatic organisms. Roche & Boge (1996) in their research explained that in vivo OH-phenol towards *Dicentrarchus labrax* using metabolic indicator indicates several problems such as hypoglycemia, low level of blood urea nitrogen (BUN), and decreased activity of alkaline phosphatase (ASP). Furthermore, Emrizal et al (2014) reported that *P. crocatum* leaves had cytotoxic activity.

Several phytochemical compounds of *P. crocatum* leaves have been reported. Craft et al (2012) showed that *P. crocatum* leaves contain compounds such as flavonoids, alkaloids, terpenoids and tannins. Phenolic compounds are the example of toxic chemicals which disrupt the endocrine system and hormones (Kuch & Ballschmiter 2001). These compounds also have great potential to interfere the immune system and increase the risk of secondary infections in fish (Writer et al 2010; Awachie & Ezenwaji 1998). Phenolic compounds are commonly found in ocean and in fish tissue, and its characteristics are chronic toxicity and chronic immunotoxicity (Mukherjee et al 1990; Taysse et al 1995).

Phenolic compound is mainly toxic compound. Cytotoxic activity of *P. crocatum* leaves (*P. crocatum*) in Artemia which is tested based on the fraction has 2.04 ug/mL of LC50 (n-hexane), 1.34 ug/mL of LC50 (ethyl acetate) and 2.08 ug/mL (fraction butanol) of LC50 Emrizal et al (2014) reported that. Phenolic compounds are difficult to be detected, due to its light taste and smell (Tilak et al 2007). Therefore phenolic compounds become concern in aquatic environment.

Clarias gariepinus is one of the fishes which live in freshwater habitat such as rivers, irrigation canals, and lakes. *C. gariepinus* faces the risk of being exposed to chemicals in nature or in farming activities which use waste water containing phenol residue for its production activity. Surprisingly, there is insufficient information on the tannin toxicology effect and pathological consequences on *C. gariepinus*. Hence, in this study we investigated the effect of polyphenol compound in *P. crocatum* leaves (*P. crocatum*) towards blood biochemistry and histopathology.

Material and Method

Isolation and characterization of poliphenol in P. crocatum leaves. *P. crocatum* leaves was obtained from herbal plants farmer in Malang, East Java. Maceration method with methanol as eluent was performed for isolating poliphenol (tannin) was done by using methanol following a protocol of Prihanto et al (2012). It was further purified based on Sundang et al (2012) method, thin-layer chromatography and column chromatography following the protocol of Bigoniya & Singh (2014) and Vasconcelos et al (2010). Polyphenol was characterized by using UV-Vis spectrophotometer and infrared along with comparison of standard tannin.

Animal testing. *C. gariepinus* from "Fish Breeding Centers" Kepanjen, Malang with the size of 10–12 gram were used for study. There were 10 fish in each aquarium. The fish was maintained in water flow system and experienced five days of adaptation prior to treatment. The range of water quality, DO, pH, and temperature was set as follow: 4.91–5.73 mg/L, 7.2–7.5, 25–26^{o}C, respectively. Commercial fish feed was applied in this research. The composition of the fish feed was: fish meal 65%, corn gluten meal 38%, soybean meal 42% and wheat meal 17%.

Fish treatment with lethal concentration extracts. Four groups (10 fish/aquarium) under control; 50% of *P. crocatum* tannin as deadly concentration was given for 96 hours. Prior to determine the lethal concentrations, we investigated it by using brine shrimp lethally test (BSLT) following the method of Firdaus et al (2013) (unpublished data). The LC50 was 0.36 mg/kg (LD 25), 1.08 mg/kg (LD50) and 1.80 mg/kg (LD 75). The exposure period of tannin compound was investigated for 3 days after the single injection. Control negative (without extract treatment) and positive control (phenol 3 mg/kg) was applied in the experiments.

Sample of blood and tissue. The samples of blood, kidney, and liver were taken after 3 days of exposure. The analysis followed the method of Roche & Boge (1996) and Varadarajan et al (2014) with slight modifications. Blood sample was taken from fish cardinal vein. It was directly centrifuged with the speed of 3000 rpm at 4^{o}C for 15 min. The serum was separated and stored in -20^{o}C until further analysis. Liver, as well as kidney were dissected and stored in 4% neutral buffered formaldehyde solution.

Blood biochemistry analysis. Measurement of hemoglobin was performed by using Sahli method (Wedemeyer & Yasutake 1977). Hemoglobin analysis was indicated on the unit of Hb/100 mL. The hematocrit measurement was performed by using capillary method (Anderson & Siwicki 1995). Then, BUN was measured by using a method from Chaney & Marbach (1962) and Searcy et al (1967). The glucose and ALP (alkaline phospatase) level were measured with colorimetric method. The concentration of plasma cortisol (Cort) was measured by using radioimmunoassay (RIA) following Foster & Dunn (1974) method.

Data analysis. The obtained data were analyzed by using Analysis of variance (ANOVA) with Minitab 14 software for Windows.

Results. The result of blood biochemical (hemoglobin, alkaline phosphatase, level of blood urea nitrogen, and level of glucose) analysis could be seen in Table 1. The highest (10.67±0.58) hemoglobin count was found in the lowest extract treatment (A treatment). It seemed that higher extract will resulted in the lower hemoglobin count. This trend was also found for ALP and glucose analysis. In contrast, the different trend was noted in BUN result. The higher of the extract the higher of the BUN.

Table 1

The analysis results of the biochemical parameters of blood (hemoglobin, alkaline phosphatase, level of blood urea nitrogen, and level of glucose)

Treatment	*Hemoglobin (Hb/100 mL)*	*ALP U/L*	*BUN (mg/DL)*	*Glucose (mg/DL)*
X	14.67±1.15	14.00±1.00	2.40±0.20	43.67±2.52
A	10.67±0.58	7.67±0.58	2.97±0.12	33.67±2.08
B	10.00±1.00	6.33±0.58	3.40±0.26	30.67±2.52
C	8.33±0.58	5.33±0.58	3.47±0.23	26.67±1.53
Y	6.67±0.58	6.33±0.58	3.40±0.20	27.00±1.00

X - Negative control (without treatment); A - poliphenol (tannin) *P. crocatum* 0.36 mg/kg; B - poliphenol (tannin) *P. crocatum* 1.08 mg/kg; C - poliphenol (tannin) *P. crocatum* 1.80 mg/kg; Y - Positive control (phenol 3 mg/kg).

The result from data analysis revealed that hemoglobin after the exposure of tannin compound was significantly different with other treatment in the standard of 5%, Fcal: 28.85 (P>0.05). Based on Table 1, the hemoglobin level in *C. gariepinus* without treatment (control group) was 14.67 hb/100 mL. Meanwhile, ALP after tannin compound exposure was significantly different with other treatment in the standard of 5%, Fcal: 91.11 (P>0.05). Based on Table 1, the ALP value in *C. gariepinus* without treatment (control group) was 12.67 U/L.

BUN after tannin compound exposure was significantly different with other treatment in the standard of 5%, Fcal: 16.42 (P>0.05). Based on Table 1, the BUN value in *C. gariepinus* without treatment (control group) was 2.4 mg/dL. Meanwhile, glucose analysis revealed that after the exposure of tannin compound was significantly different with other treatment in the standard of 5%, Fcal: 14.84 (P>0.05).

Kidney alteration. Necrosis and congestion was found after phenolic injection. Histopathology analysis of kidney was depicted in Figure 1. Necrosis and congestion of kidney tissue was occurred in the treatment of *P. crocatum* extract and positive control.

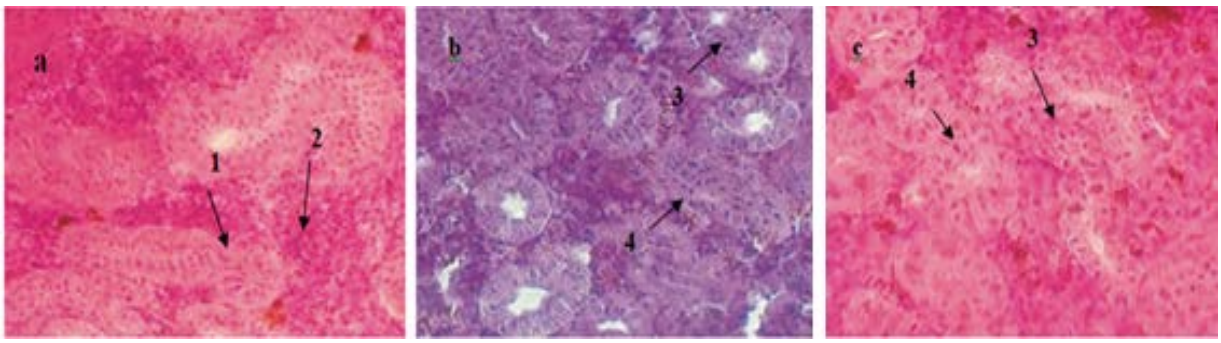

Figure 1. Renal histopathology of *Clarias gariepinus*. (a) normal kidney (control), (b) exposure with tannin, (c) exposure with phenol. Arrow No. 1. The distal tubule (T); 2. Hematopoietic network (HN); 3. Necrosis; 4. Congestion (400 x magnifications by light microscopy).

It was further observed by using scoring analysis. The mean result of the viewing area of kidney tissue was presented in Table 2.

The average value of the necrosis analysis indicated that the treatment analysis in the concentration of 1.80 mg/kg contributed the same result with positive control. In case of necrosis, treatment of 1.8 mg/kg was the lowest concentration of tannin to give the same effect as a positive control (3 mg/kg). Meanwhile, congestive tissue of kidney was also occurred in the treatments. Control positive contributed to the worst congestive in kidney tissue. In the concentration of 1.80 mg/kg, the treatment showed lower size. Hence, the treatment indicated slight different effect on necrosis and congestion of kidney tissue.

Table 2

The average value of necrosis and congestive view size in kidney tissue

Abnormalities histology	*Treatment*	*Average of view size*
Necrosis	Negative control (without treatment)	1
	Tannin (1.80 mg/kg)	2.2
	Positive control (3 mg/kg)	2.2
Congestive	Negative control (without treatment)	1
	Tannin (1.80 mg/kg)	1.8
	Positive control (3 mg/kg)	2

Liver alteration. After phenolic injection the liver tissue experienced damage such necrosis and hypertrophy. It was then observed by using scoring assessment. Figure 2 represented the normal liver tissue, the liver tissue which was exposed to tannin, and the liver tissue which was exposed to phenol.

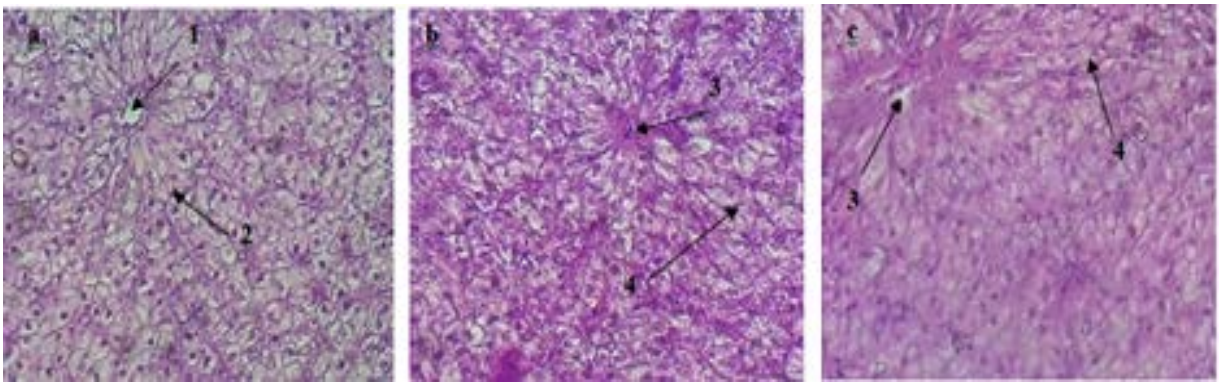

Figure 2. Liver histopathology of *Clarias gariepinus*. (a) normal liver (control), (b) exposure with tannin, (c) exposure with phenol. Arrow No. 1. The distal tubule (T); 2. Sinusoid (S); 3. Necrosis; 4. Hypertrophic (400 x magnifications by light microscopy).

The average size of the area of liver tissue which experienced necrosis, as well as hypertrophy could be seen in Table 3. Under the tannin exposure of 1.80 mg/kg, the damage of the tissue exhibited lower size than that of positive control (30 mg/kg). Contrastingly, in the hypertrophy, similar effect was shown by treatment and positive control with the average size of 3.2.

Table 3

The average value of necrosis and hypertrophy view size in liver tissue

Abnormalities histology	*Treatment*	*Average of view size*
Necrosis	Negative control (without treatment)	1
	Tannin (1.80 mg/kg)	2.2
	Positive control (3 mg/kg)	3.2
Hypertrophy	Negative control (without treatment)	1
	Tannin (1.80 mg/kg)	3,2
	Positive control (3 mg/kg)	3,2

Discussion. Blood analysis indicated that the hemoglobin is reduced by treatments. Hemoglobin level in healthy *C. gariepinus* is in the range of 12-14 Hb/100 mL Alamanda et al (2007). In several report, an addition of phenolic compounds on the fish, will reduce the amount of hemoglobin. Study by Varadarajan et al (2014) revealed that giving phenolic compound to *Oreochromis mossambicus* will also decrease the hemoglobin. This is due to the enlargement and/or damage of red blood cell (Harikrishnan et al 2003). However, some contrast results were reported by Roche & Boge (1996) which reported the increase of hemoglobin concentration after being injected with phenol.

ALP activity on the blood could be affected by several factors such as age, weight, and species. The normal ALP on fish should be about 30-60 U/L, even though the value of blood biochemistry can be different for each species. This result was similar with the study of Roche & Boge (1996) which explained that phenolic injection to *D. labrax* fish would decrease ALP. It is caused by nephrotoxicity and elimination (Peters et al 1997).

BUN value was relatively diverse which was caused by various factors such as age, weight, and species. However, fish normally has low BUN value (Hrubec et al 1997). Compared to *D. labrax* with the range of BUN in the range of 9-12 U/L, BUN in this study was low. The increase of BUN value was possibly because of the reduction of kidney's cells function. The change in BUN value after phenolic exposure was presumed to be the effect in protein metabolism (Gupta et al 1983).

Based on Heath (1987), normal blood glucose level in *C. gariepinus* was in the range of 41-150 mg/dL. Varadarajan et al (2014) reported that phenol exposure in *O. mossambicus* fish shows a decrease of glucose level due to disorder of metabolism, homeostasis of blood glucose, and hormone. The decrease of glucose could also be induced by chronic stress of fish. The result of this stress is hypoglycemia (Hrubec et al 1997).

Necrosis was found in all organs. Necrosis is a condition where tissue cells experience premature death, it begins with low activity in the tissue which leads to malfunction of infected cells (Price & Wilson 2006; Maftuch et al 2016). Liver and kidney were the main organs to detoxify organic xenobiotic. Liver damage due to phenol exposure was in line with study of Abdel-Hameid (2007) which found that phenol concentration in body water caused organs alteration. Phenolic addition to tannin and phenol treatment resulted in moderate tissue damage as shown in scoring assessment. This alteration causes disorder of endocrine organs. Hence, it might interfere the metabolism of organism, thus the organ could not function normally. This phenomenon was also noted by McKim et al (1999). *Oncorhynchus mykiss* which has been exposed to phenolic compounds showed damage of renal tubular epithelia. The disorder of kidney was responsible to filter and excrete materials which were unnecessary for the body, including compounds and toxic heavy metals. The toxic compounds or metals contribute to the kidney damage (Fitriawan et al 2011).

C. gariepinus shows abnormal sign such as liver necrosis after being exposed to phenolic compound (Ibrahem (2012). Liver is the main organ for detoxification of organic xenobiotics which causes proportional changes to the liver as the result of phenol exposure Abdel-Hameid (2007). It was note that the exposure of tannin could trigger organs alteration.

Conclusions. The treatment of tannin on fish affected blood biochemistry and several organs alterations. Blood biochemistry, hemoglobin, blood urea nitrogen (BUN), glucose, and alkaline phosphatase (ALP) decreased. Kidney damage in the form of necrosis could be seen from kidney and liver histopathology tests. Hence, it was obvious that the exposure of tannin compound with different doses had different effects towards the blood biochemistry and organs tissues of *C. gariepinus*.

References

Abdel-Hameid N.-A. H., 2007 Physiological and histopathological alterations induced by phenol exposure in *Oreochromis aureus* juveniles. Turkish Journal of Fisheries and Aquatic Sciences 7:131–138.

Alamanda I. E., Handajani N. S., Budiharjo A., 2007 [Use of hematology and observation method for determination of blood endoparasit health Dumbo Catfish (*Clarias gariepinus*) in pond aquaculture Mangkubumen Village]. Boyolali Biodiversitas 1:34-38. [In Indonesian].

Ali S. M., Sabae S. Z., Fayez M., Monib M., Hegazi N. A., 2011 The influence of agro-industrial effluents on River Nile pollution. Journal of Advanced Research 2:85–95.

Anderson D. P., Siwicki A. K., 1995 Basic hematology and serology for fish health programs: Fish Health Section, Asian Fisheries Society, pp. 185-202.

Awachie J. B. E., Ezenwaji H. M. G., 1998 The importance of *Clarias* species in the fishery development of Anambra River Basin. CIFA Tech Paper 8:212–224.

Bigoniya P., Singh K., 2014 Ulcer protective potential of standardized hesperidin, a citrus flavonoid isolated from *Citrus sinensis*. Revista Brasileira de Farmacognosia 24:330-340.

Chamidah A., Prihanto A. A., Hardoko H., 2017 Edema-reducing activity of *Sargassum crassifolium* β-glucan (Laminaran) on edema-induced *Rattus norvegicus*. Asian Journal of Pharmaceutical & Clinical Research 10:311-313.

Chaney A. L., Marbach E. P., 1962 Modified reagents for determination of urea and ammonia. Clinical Chemistry 8:130-132.

Craft B. D., Kerrihard A. L., Amarowicz R., Pegg R. B., 2012 Phenol based antioxidants and the in vitro methods used for their assessment. Comprehensive Reviews in Food Science and Food Safety 11:148–173.

Dewi Y. F., Anthara S. M., Dharmayudha A. G. O., 2014 [Effectivity of *Piper crocatum* on the reduction of glucose in alloksan-induced rat (*Rattus novergicus*)]. Bulettin Vetereiner Udayana 6(1):75-78. [In Indonesian].

Emrizal, Fernando A., Yuliandri R., Rullah K., Indrayani N. R., Susanty A., Yerti R., Ahmad F., Sirat H. M., Arbain D., 2014 Cytotoxic activities of fractions and two isolated compounds from Sirih Merah (Indonesian red betel), *Piper crocatum* Ruiz and Pav. Procedia Chemistry 13:79–84.

Firdaus M., Prihanto A. A., Nurdiani R., 2013 Antioxidant and cytotoxic activity of *Acanthus ilicifolius* flower. Asian Pacific Journal of Tropical Biomedicine 3:17–21.

Firdaus M., Prihanto A. A., Nurdiani R., 2015 Antihyperglycemic of *Sargassum* sp. Extract. Marine algae extracts: processes, products, and applications. Pp. 381-393. Wiley-VCH Verlag GmbH & Co. Weinheim, Germany.

Fitriawan F., Sutarno, Sunarto, 2011 Microanatomy alteration of gills and kidneys in freshwater mussel (anodonta woodiana) due to cadmium exposure. Nusantara Bioscience 3:28-35.

Foster L. B., Dunn R. T., 1974 Single antibody technique for radioimmunoassay of cortisol in unextracted serum or plasma. Clinical Chemestry 20:365–368.

Gupta S., Dalela R. C., Saxena P. K., 1983 Effect of phenolic compounds on in vivo activity of transaminases in certain tissues of the fish, *Notopterus notopterus*. Environmental Research 32:8–13.

Harikrishnan R., Rani M. N., Balasundaram C., 2003 Haematological and biochemical parameters incommon carp, *Cyprinus carpio*, following herbal treatment for *Aeromonas hydrophilla* infection. Aquaculture 221:41-50.

Harjana Tri 2011 Study on the potential of natural materials to reduce blood cholesterol levels. Proceeding of National Conference on Research, Pendidikan dan Penerapan MIPA. Fakultas MIPA, Yogyakarta National University, p. 4.
Heath A. G., 1987 Water pollution and fish physiology. CRC press, Florida, USA, 245 pp.
Hrubec T. C., Robertson J. L., Smith S. A., 1997 Effects of temperature on hematologic and serum biochemical profiles of hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). American Journal of Veterinary Research 58:126–130.
Ibrahem M. D., 2012 Experimental exposure of African catfish *Clarias gariepinus* (Burchell, 1822) to phenol: clinical evaluation, tissue alterations and residue assessment. Journal of Advanced Research 3:177–183.
Kuch H. M., Ballschmiter K., 2001 Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC−(NCI)−MS in the picogram per liter range. Environmental Science and Technology 35(15):3201–3206.
McKim J. M., Kolanczyk R. C., Lien G. J., Hoffman A. D., 1999 Dynamics of renal excretion of phenol and major metabolites in the rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 4:265–277.
Maftuch, Aziz K., Eslfitri D., Sanoesi E., Prihanto A. A., 2016 Enrofloxacin stimulates cell death in several tissues of vannamei shrimp (*Litopenaeus vannamei*). Comparative Clinical Pathology doi:10.1007/s00580-016-2384-0.
Mukherjee D., Bhattacharya S., Kumar V., Moitra J., 1990 Biological significance of [14C] phenol accumulation in different organs of a murrel, *Channa punctatus*, and the common carp, *Cyprinus carpio*. Biomedical and Environmental Sciences 3:337–342.
Peters M. M., Jones T. W., Monks T. J., Lau S. S., 1997 Cytotoxicity and cell-proliferation induced by the nephrocarcinogen hydroquinone and its nephrotoxic metabolite 2,3,5-(tris-glutathion-S-yl) hydroquinone. Carcinogenesis 12:2393–2401.
Price S. A., Wilson L. M., 2006 Pathophysiology and clinical concept of disease. 6th Edition, EGC, Jakarta, 1570 pp.
Prihanto A. A., Firdaus M., Nurdiani R., 2012 Anti-methicillin resistant *Staphylococcus aureus* (MRSA) of methanol extract of mangrove plants leaf: preliminary report. Drug Invention Today 4:439–440.
Roche H., Boge G., 1996 Fish blood parameters as a potential tool for identification of stress caused by environmental factors or chemical intoxication. Marine Environmental Research Part C 125:27–44.
Searcy R. L., Reardon J. E., Foreman J. A., 1967 A new photometric method for serum urea nitrogen determination. The American Journal of Medical Technology 33:15-20.
Sundang M., Nasir S. N. S., Sipaut C. S., Othman H., 2012 Antioxidant activity, phenolic, flavonoid and tannin content of Piper betle and Leucosyke capitella. Malaysian Journal of Fundamental and Applied Sciences 8(1):1-6.
Taysse L., Troutaud D., Khan N. A., Deschaux P., 1995 Structure activity relationship of phenolic compounds (phenol, pyrocatechol and hydroquinone) on natural lymphocytotoxicity of carp (*Cyprinus carpio*). Toxicology 1-3:207–214.
Tilak K. S., Veeraiah K., Butchiram M. S., 2007 Effect of phenol on haematological components of Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. Journal of Environmental Biology 2:177–179.
Varadarajan R., Sankar H. S., Jose J., Philip B., 2014 Sub-lethal effects of phenolic compounds on biochemical, histological and ionoregulatory parameters in a tropical teleost fish *Oreochromis mossambicus* (Peters). International Journal of Scientific and Research Publications 3:2250-3153.
Vasconcelos P. C. P., Andreo M. A., Vilegas W., Hiruma-Lima C. A., Pellizzon C. H., 2010 Effect of *Mouriri pusa* tannins and flavonoids on prevention and treatment against experimental gastric ulcer. Journal of Ethnopharmacology 131:146–153.
Wedemeyer G. A., Yasutake W. T., 1977 Clinical methods for the assessment of the effects of environmental stress on fish health. Technical Paper of the U.S. Fish and Wildlife Service. Vol. 89. U.S. Department of the Interior Fish and Wildlife Service, Washington, D.C., USA, p. 18.

Writer J. H., Barber L. B., Brown G. K., Taylor H. E., Kiesling R. L., Ferrey M. L., 2010 Anthropogenic tracers, endocrine disrupting chemicals and endocrine disruption in Minnesota lakes. The Science of the Total Environment 1:100–111.

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