Journal of Research in Ecology

An International Scientific Research Journal

ISSN No: Print: 2319 –1546; Online: 2319– 1554

Short Communication

Determination of the toxicity of methanol extraction of red betel leaves (*Piper crocatum* Ruiz & Pav) on African sharp tooth catfish (*Clarias gariepinus*)

Authors:
Afandi Saputra[1] and Septyan Andriyanto[2]

Institution:
1. Aquaculture Department, Jakarta Fisheries University, Indonesia

2. Research Institute For Freshwater Aquaculture, Bogor, Indonesia

Corresponding author:
Afandi Saputra

Email ID:

afandi.saputra@yahoo.co.id

ABSTRACT:

Reports have mentioned that red betel leaf (*P. crocatum*), a type of vegetable product, functions as anti-stress, growth promotor, appetite stimulation, imunostimulant, as well as antimicrobial for fish because red betel leaf contains alkaloids, tannins, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oils. The objective of the study was to describe isolation of tannin class compounds using characteristics of UV-Vis and FTIR spectrophotometer method as well as LC_{50} score of *P. crocatum* tannin concentration in African sharptooth fish (*C. gariepinus*) as treatment agent. The extraction method used in the study was maceration in which methanol became the solvent. The sample was dissolved for 48 hours and ratio between the sample and solvent was 1: 2.5 (b:v). Thin Layer and Column Chromatography were used for isolating the tannin class compounds. The isolation of the tannin class compounds in the red betel leaves using the characteristics of UV-Vis and FTIR Spectrophotometer showed that isolated compound was the tannin class compound. Based on the LC_{50} toxicity testing of the *P. crocatum* tannin concentration, the highest score occurred when the concentration was 2.16 mg/kg.

Keywords:

Clarias gariepinus, LC_{50}, Piper crocatum, tannin

Article Citation:
Afandi Saputra and Septyan Andriyanto
Determination of the toxicity of methanol extraction of red betel leaves (*Piper crocatum* Ruiz & Pav) on African sharp tooth catfish (*Clarias gariepinus*)
Journal of Research in Ecology (2017) 5(2): 1141-1147

Dates:
Received: 21 July 2017 **Accepted:** 10 Aug 2017 **Published:** 24 Sep 2017

Web Address:
http://ecologyresearch.info/documents/EC0492.pdf

Journal of Research in Ecology

INTRODUCTION

Natural extract is one of many resources that may be used for curative treatment or medicine for fish and so, further exploration is conducted. It has been reported that vegetable products may function as anti-stress, growth promotor, appetite stimulation, imuno stimulant, as well as antimicrobial for fish due to alkaloids, tannins, flavonoids, pigments, phenolics, terpenoids, steroids, and essential oils they contain (Sivaram *et al*., 2004; Zhang *et al*., 2009). Herbal immune stimulants can modulate the innate immune response that are alternative for cost-effective antibiotics and solution towards the limited supply of antibiotics, chemicals or medicines currently used to treat fish (Fu *et al*., 2007; Valentim *et al*., 2008). Immuno stimulants are extraordinarily successful and eco-friendly in approach to fish disease management (Peddie *et al*., 2002; Cruz *et al*., 2008; Fujimoto *et al*., 2012).

Fifteen steroids, 18 kavapyrones, 17 chalcones/ dihydrochalcones, 16 flavonesOne of the plants from Indonesia that can potentially be used as medicine is red betel leaf (*Piper crocatum*). In the genus Piperaceae, 677 different compounds have been isolated from 112 species. There are 190 alkaloids/amides, 49 lignans, 70 neolignans, 97 terpenes, 39 phenylpropanoids, 6 flavanones, 4 piperolides (cinnamylidone butenolides) and 146 other compounds that cannot be categorized as the general large group of secondary metabolites (Dyer *et al*., 2004). Craft *et al*., (2012) stated that red betel leaves have chemical content with certain properties called secondary metabolites that store active compounds such as flavonoids, alkaloids, terpenoids, cyanogenic, glucoside, isoprenoids, nonprotein amino acids, and eugenol. On the other hand, flavonoid and polevenolad compounds contain antioxidant, antidiabetic, anticancer, antiseptic, and anti-inflammatory properties.

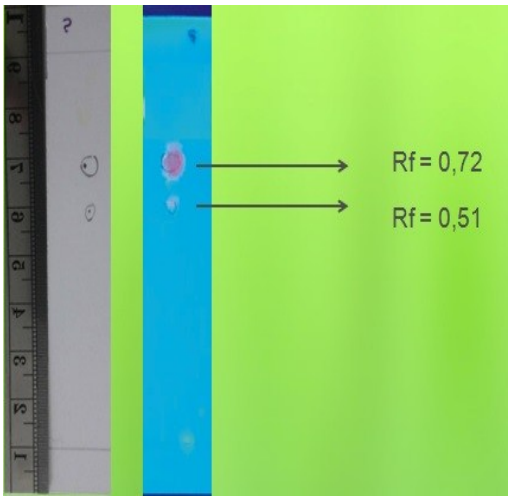

Figure 1. TLC result of red betel leaf extract (*P. crocatum*)

Red betel (*Piper crocatum*) is an alternative medicine people widely use. Craft *et al*. (2012) explained that red betel leaves contain compounds such as flavonoids, alkaloids, terpenoids, and tannins. Flavonoids and tannins are classified as polyphenolic, in which flavonoid has 15 carbon atoms consisting of two benzene rings connected together by a linear chain comprising three carbon atoms (Markham, 1982). Syahida *et al*., (2013) reported the addition of red betel leaf extract (*P. crocatum*) with various doses of fishfeed that has significant effect on total erythrocytes, total leucocyte, lymphocyte percentage, monocyte percentage and phagocytosis index for carp (*C. carpio*) after *Aeromonas hydrophila* challenge test. Based on those information, in order to develop medication or curative treatment for fish, a study describing LC_{50} tannin concentration in *P. crocatum* towards African sharp-tooth fish (*Clarias gariepinus*) is of neccessity.

MATERIALS AND METHODS

Research method

The study lasted for three months (Maret – Mei 2017) in the Chemistry Laboratory of State Islamic University, Maulana Malik Ibrahim Malang, and Environment Laboratory of Higher Institute of Fisheries (*STP/ Sekolah Tinggi Perikanan*)

Testing animal

The testing animals were African sharp tooth

catfish (*Clarias gariepinus)* that weighed between 10 and 12 grams; there were 10 fish/ tub. Water maintenance was replied with floating water system. The parameters for the quality of water were DO of 4.91- 5.73 mg/l, pH of 7.2-7.5 and temperature of 25-26 ^{o}C. *Ad libitum* fish feeding with commercial fish feed was started two weeks prior to the experiment.

Isolation and characteristics of red betel leaf tannin

The betel leaves were harvested from herbal nursery in Malang. The methods used for isolating the *P.crocatum* tannin were maceration followed by the Thin Layer (TLC) and Column chromatography. The results were characterized using UV-Vis and FTIR spectrophotometer and then compared to the standardized tannins.

Tannin extraction of red betel leaf

Sundang *et al.* (2011)'s method was adopted for extracting tannin form the red betel leaves while Bigoniya and Singh **(**2014) and Vasconcelos *et al.* (2010)'s method was adopted for the Thin Layer and Column chromatography. In order to get phenolic extraction form the red betel leaves, 100 grams of red betel leaf powder (wet, wilted and dried) were poured into a 500-milliliter beaker glass. 250 milliliters of methanol was poured into the glass to dissolve the powder. The solvent was homogenized using magnetic stirre for 30 minutes.

Thin layer and column chromatography

In order to separate red betel leaf tannin, the column chromatography with a ration between methanol and ethyl acetate of (1:8, v/v) was used to separate tannin class compounds. Prior to separating tannins using the column chromatography, the (TLC) was conducted for obtaining Retention factor (R_f) score for identifying tannin area in silica gel.

Identifying Tannin Compound using UV-Vis and FTIR Spectrophotometer

UV-Vis spectrophotometer was used for identifying tannin class compounds from the red betel leaves. The first step was to pour three millilitre of the sample obtained from the column chromatography into the cuvette. Next, the spectrophotometer was scanned with the

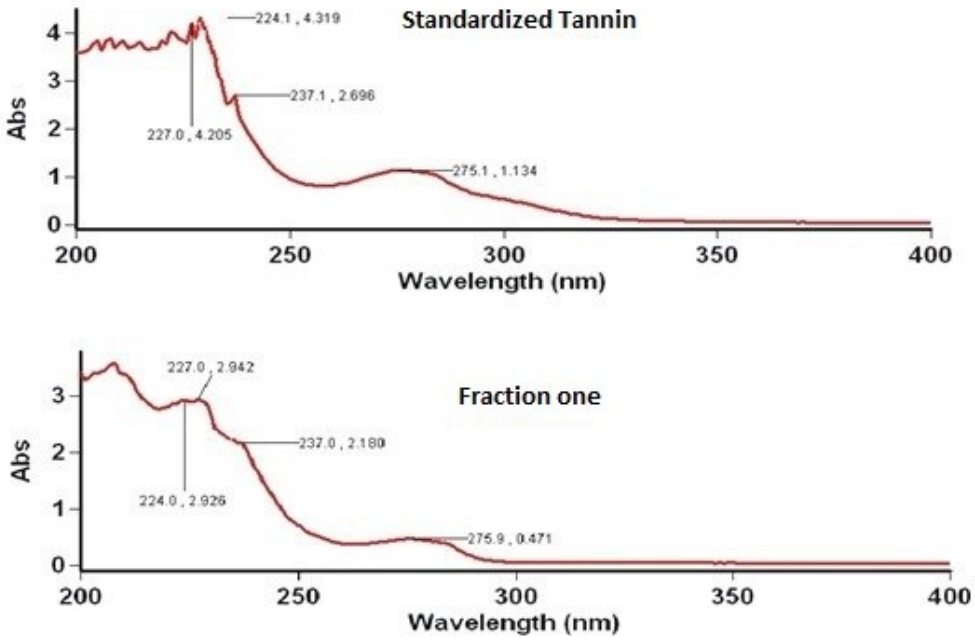

Figure 2. Identification of tannins in fraction one (I) compared to the standardized tannin using UV –Vis Spectrophotometer

Table 1. LC_{50} of *Piper crocatum* Ruiz and Pav tannin in the African sharp tooth catfish for 96 Hours

Dosage of Injection	Mortality±SD (%)
0.72 mg/kg	3.33±5.77
1.08 mg/kg	6.67±5.77
1.44 mg/kg	13.33±5.77
1.80 mg/kg	23.33±5.77
2.16 mg/kg	53.33±5.77

wavelength between 200 nm and 550 nm and calibrated using methanol. The cuvet filled with tannin filtrate was put inside the spectrophotometer and was scanned with the wavelength between 200 nm and 550 nm. $E^{1\%}_{1cm}$ equation was used to obtain amount of the tannin (µg/gr).

Sub-lethal toxicity study

The 10 to 12 gram fish that had been adapted previously was divided into six groups with three replications (10 fish/tub). 50% of lethal concentration of *P. crocatum* tannin within 96 hours was exposed into single injection of which dosage was 0.36, 0.72, 1.08, 1.44, 1.80 and 2.16 mg/kg.

RESULTS

Isolation and characteristics of red betel leaf tannin

Isolation of tannin using Thin Layer (TLC) and column chromatograph

Figure 1 described the result of the Thin Layer (TLC) and column chromatograph of dried red betel leaf extract under UV light with the wavelength between 256 nm and 366 nm.

Identification of tannin compounds using UV-Vis and FTIR spectrophotometer

Figure 2 described the result of tannin identification in the fraction compared to the standardized tannin using UV -Vis spectrophotometer.

Identifying tannin compound using FTIR spectrophotometer

Figure 3 described the result of tannin compound identification in fraction one using the FTIR spectrophotometer.

Sub-lethal (LC_{50}) Toxicity Study

Toxic activity testing using African sharptooth catfish (*C. gariepinus*) in order to detect the toxic concentration of *Piper crocatum* Ruiz & Pav tannin lasted for 96 hours. Table 1 described the LC_{50} of the tannin in the *P. crocatum*.

DISCUSSION

Isolation and characteristics of red betel leaf tannin

Isolating tannin compound using TLC and column chromatography

Based on the result of the TLC, eluent methanol: ethyl acetate (1:8 v/v) resulted in 2 isolates in the TLC plate. In Figure 3, the TLC plate after elution showed that the R_f scores were 0.72 and 0.51. The tannin was found in the Isolate 2 in which the R_f score was 0.72 which was in line to the comparison between the wavelength of the standardized tannin and that of the isolate 2. The color of the tannin compound was pink under the UV light where the wavelength was 256. It is important to determine eluent in isolating small-scale pure compound because the more polarized and accurate mixture between solvent was, better isolation, the pure compound we received. Tyson (1988) stated that process of determining eluent was an alternative for pure compound isolation.

Having finished determining the eluent in TLC, the following step in isolating tannin class compound in *P. crocatum* was to conduct column chromatography based on Bigoniya and Singh (2014) and Vasconcelos *et al.* (2010)'s method. The chromatography resulted in four fractions and the UV-Vis spectrophotometer was run for each of the fractions in order to describe the wavelength compared to the standardized tannin. Fraction one and the standardized tannin had similar wavelength.

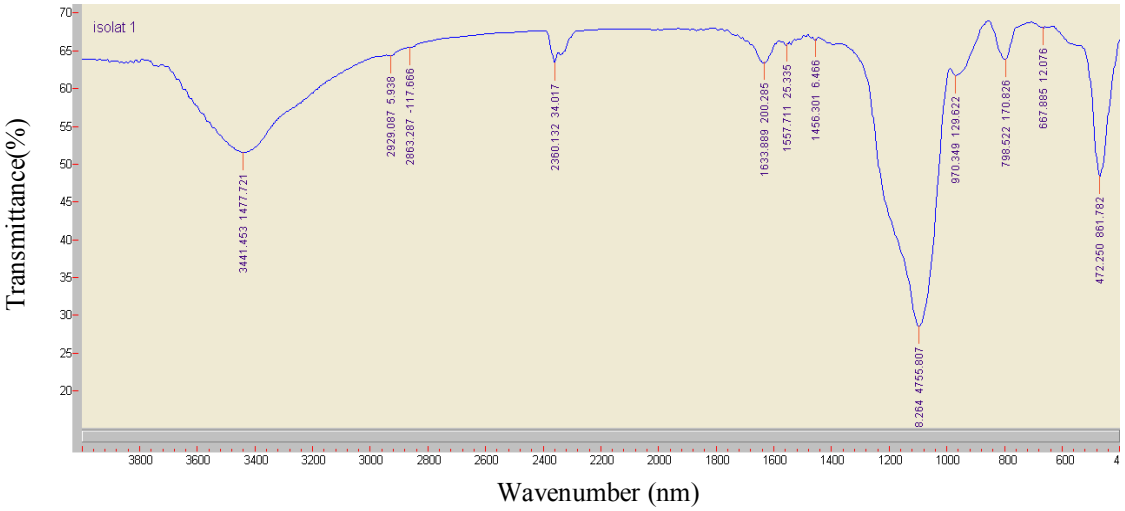

Figure 3. Identification of tannin compounds in fraction one (I) using FTIR

Identifying tannin compound using UV-Vis spectrophotometer

Qualitatively speaking, absorbance of the fraction one of *P. crocatum* showed that there were four types of wavelength described in Figure 2. The wavelength in fraction one is similar to that of the standardized tannin. The wavelength in UV-Vis spectra were 224 nm, 227 nm, 237 nm and 275 nm. Harbone (1987) reported the wavelength of polyphenol compound in UV-Vis spectra were between 200 and 400 nm.

Identifying Tannin Compound using FTIR Spectrophotometer

Figure 3 showed that fraction one of *P. crocatum* had several functional groups in specific absorption area, namely hydroxy group (OH) characterized by the uptake of 3440 cm^{-1}, aliphatic C-H group spans in the 2929 cm^{-1} absorption area, and C = C aromatic group in the 1637 cm^{-1} absorption area. Hagerman, (2002) stated that tannins are polymers of flavonoids connected to C 8 through C4. Gafur, et.al (2013) added that flavonoid compounds indicated specific functional groups, aliphatic C-H hydroxyl group and C = C aromatic group.

Sub-lethal (LC_{50}) Toxicity Study

Analysis towards toxicity of tannin compound in *P. crocatum*, as mentioned in Table 2 showed that 2.16 mg/kg was the highest dosage for LC_{50} delimiter. Mortality of the concentration was 53.33±5.77% and the score kept decreasing following the concentration. It explained that the suitable concentration for using the *P. crocatum* tannin as treatment agency was ≤ 1.80 mg/kg. Emrizal, *et al*., (2014) reported cytotoxic activity of red betel leaf on artemia tested based on fraction and isolation of two compounds of which LC_{50} concentration was 27.40 ± 2.6 ug /ml (methanol). Based on the LC_{50}, the plant potentially contained a cytotoxic compound.

CONCLUSION

Based on the isolation of red betel leaf tannin compound using UV-Vis and FTIR spectrophotometer, the isolated compound is a tannin class compound. Toxicity testing towards the *P. crocatum* tannin in the African sharptooth catfish showed that the highest LC_{50} occurred when the concentration was 2.16 mg/kg. It showed that ≤ 1.80 mg/kg was the safest concentration for the use of *P. crocatum* tannin as a treatment agent.

REFERENCES

Bigoniya P and Singh K. (2014). Ulcer protective potential of standardized hesperidin, a citrus flavonoid isolated from *Citrus sinensis. Revista Brasileira de Farmacognosia*, 24(3): 330-340.

Craft BD, Adrian LK, Ryszard A and Ronald BP. (2012). Phenol based antioxidants and the *in vitro* methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety*, 11(2): 148–173.

Cruz C, Machado NJG, Fujimoto RY, Henares MNP and Duo DA. 2008. Eficacia do paration metílico e do extrato aquoso de folhas secas de nim no controle de Anacanthorus penilabiatus (Monogenoidea) em pacu (*Piaractus mesopotamicus*). *Boletim do Instituto de Pesca,* 34(1):61-69.

Dyer LA, Richards J and Dodson CD. (2004). Isolation, synthesis, and evolutionary ecology of piper amides. In: Piper: A model genus for studies of phytochemistry, ecology and evolution. *Kluwer Academic/ Plenum Publisher New York*; 117-39.

Emrizal, Fernando A, Yuliandri R, Rullah K, Nola Indrayani NR, Susanty A, Yerti R, Ahmad F, Sirat HM and 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.

Fu YW, Hou WY, Yeh ST, Li CH, Chen JC. (2007). The immunostimulatory effects of hotwater extract of *Gelidium amansii* via immersion, injection and dietary administrations on white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. *Fish and Shellfish Immunology*, 22(6): 673-85.

Fujimoto RY, Costa HC, Ramos FM. (2012). Controle alternativo de helmintos de Astyanax cf. zonatus utilizando fitoterapia com sementes de abobora (*Cucurbita maxima*) e mamao (*Carica papaya*). *Pesqui Vet Bras,* 32:5-10.

Gafur MA, Isa I and Bialangi N. (2013). Isolasi dan identifikasi senyawa flavonoid dari daun jamblang (*Syzygium cumini*). Faculty of Mathematics and Natural Science. State University of Gorontalo, Gorontalo. 11p.

Hagerman AE. (2002). Tannin Chemistry. Miami (US): Miami University, 1-7p.

Harbone JB. (1987). Metode Fitokima : Penuntun cara modern menganalisa tumbuhan. Second Edition, Bandung ITB, 345-354p.

Markham KR. (1982). Techniques of flavonoid identification. Academic Press, London.113p.

Sivaram V, Babu MM, Citarasu T, Immanuel G, Murugadass S and Marian MP. (2004). Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. *Aquaculture*, 237(1-4):9-20.

Syahida IEA, Sarjito SB, Prayitno SB and Mariana AL. (2013). Pengaruh ekstrak daun sirih merah (*Piper crocatum*) terhadap profil darah dan kelulusanhidup ikan mas (*Cyprinus carpio*) yang diinfeksi bakteri *Aeromonas hydrophila. Journal of Aquaculture Management and Technology*, 2(4):94-107.

Peddie SJ, Zou CJ. Secombes. (2002). Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. *Veterinary Immunology and Immunopathology,* 86(1-2):101-113.

Sundang M, Nasir SNS, Sipaut CS and Othman H . (2012). Antioxidant activity, phenolic, flavonoid and tanin content of *Piper betle* and *Leucosyke capitella. Malaysian Journal of Fundamental and Applied Sciences,* 8(1):1-6.

Julian Tyson. (1988). Analysis-what analytical chemists Do. CRC Press, ISBN 0851864635.

Valentim MZL, Vargas RPR. Ribeiro R, Piau MBA, Torres M, Ronnau M and Souza JC. (2008). Effects of a homeopathic complex in Nile tilapia (*Oreochromis*

niloticus L.) on performance, sexual proportion and histology. *Homeopathy*, 97(4):190-195.

Vasconcelos PC, Andreo MA, Vilegas WO, Hiruma -Lima CAO and Pellizzon CHO. (2010). Effect of *Mouriri pusa* tanins and flavonoids on prevention and treatment against experimental gastric ulcer. *Journal of Ethnopharmacology* 131(1):146–153.

Zhang GS, Gong S, Yu D and Yuan H. 2009. Propolis and herba epimedii extracts enhance the non-specific immune response and disease resistance of Chinese sucker, *Myxocyprinus asiaticus*. *Fish and Shellfish Immunology*, 26(3): 467-472.