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Short communication
Phenol contaminant of Bengawan Solo River and characteristics of *Portulaca* using flavonoid, saponin, and tannin for phytoremediation purposes

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Abstract. *Khoiriyah S, Gravitiani E, Setyono P, Suranto. 2019. Short communication Phenol contaminant of Bengawan Solo River and characteristics of* Portulaca *using flavonoid, saponin, and tannin for phytoremediation purposes. Biodiversitas 20: 3269-3274.* Bengawan Solo River (BSR) as one of the largest river in Java has been contaminated by several waste products including industrial activities and organic materials produced by households along the edge of river. One of the very dangerous substances was Endocrine Disrupting Compound (EDC). This estrogenic waste solution has been considered to have a negative impact on the environmental conditions which could cause quality water becoming worse. The aims of this research were to identify the quality BSR whether or not contaminated by phenol and to reduce the very potential pollutants of EDC by introducing local native plants of *Portulaca* species as a candidate of phytoremediation plant agents. To characterize the three species of *Portulaca*, i.e. *P. oleracea*, *P glandiflora*, and *P. pillosa*, TLC-test to detect their flavonoid, saponin, and tannin were conducted respectively. Accordingly, HPLC was used to detect the presence of EDC. The results showed that BSR's EDC has been detected with a concentration of 0,0013 ng. Also, all three species of *Portulaca* contained flavonoid, tannin, and saponin, in which the highest concentration of saponin has been detected in *P. pillosa*. Based on these results, it is expected that plants containing the highest secondary metabolism, such as saponin would be very promising to be used for phytoremediation purposes. The preliminary result of HPLC showed supporting this prediction.

Keywords: Bengawan Solo River, estrogenic, EDC, phytoremediation, *Portulaca*

INTRODUCTION

Bengawan Solo River (BSR) has been reported as the longest river (548,53 km) in Java Island, Indonesia and the area of the water movement was not only in Central Java, but also to East Java. The usefulness of this river for agricultural activities as evidence for people living along the edges until the end of the river downstream. Many advantages of this river existence such as drinking water were believed occurred long time ago. But in recent years, many industrial activities, as well as family household wastes had resulted in the quality of BSR getting worse**.**

This waste product was very potent in reducing the water quality of BSR. One of the very dangerous substances was Endocrine Disrupting Compound (EDC), such as phenol. It eventually could cause disrupting the function of the reproductive hormone (estrogen). This EDC was very stable and could not be degraded in physic, biologic or even chemistry and could be accumulated in lipid, the tissue of human beings or even in the animal or plant within a long time. This high concentration of phenol (0,087-1,431 mg/L) has been reported by Utomo et al. (2017). Also, Ben (2015) reported that almost all surface waters always contaminated by EDC. This phenolic waste product could disrupt the function of endocrine hormone and therefore could be very harmful to women (Wirasnita 2018). This substance usually contained in batik coloring material (Kulkarni and Kaware 2013). The phenolic waste product has been noted to be relatively toxic, corrosive and very dangerous when present in very high amount in the water and could cause concentration of O_2 becoming dropped (Karci 2014). This occurrence would result in the water ecosystem disturbed, including plant, animal and very possibly the aerobe bacteria could be died (Khusnuryani 2015). Although this phenolic substance in the water solution was very dangerous, Fenton reagent could be used to degrade it by using the oxidation process (Setiyaningsih 2018).

A previous study concerning the quality of BSR, particularly in the area of Solo-Sragen indicated that the quality of water has been recorded very bad due to the high concentration of phenol (0.0087-1.413 mg/L) (Utomo et al. 2017). This very bad quality of BSR would eventually give a negative impact on the drinking water of Surakarta peoples because their drinking water was made up of the BSR water. This water is a natural source which is very

potential to be used for domestic needs, raw water material for drinking water and industry, as well as irrigation (BBWS 2009).

More chemical treatments are needed when drinking water was treated when the BSR's water contained a lot of pollutants (Mulyanto 2007). Meanwhile, based on Government Regulation Republic of Indonesia No 82/2001 about water quality management and wastewater control, that water standard quality of phenol was 1 µg/L. It is very easy to detect this substance when the surface water was phenol contamination. The characteristic of water phenol contamination was the smell that is coming up from the water surface and sometimes also by the death of the organisms in the water. Therefore it is easy to understand that if that bad quality of water was consumed by a human being, it could cause several health problems, such as disrupting the function of lung, ren or even could cause the failure of blood circulation or breathing which could end by death (Kulkarni and Kaware 2013).To overcome the above problems, especially in lowering the phenol concentration in the BSR, so that the water would be safe for human consumption and good to the environmental conditions, Phytoremediation using local native plants will be conducted (Gami et al. 2014). One of the very easy collected plants which are considered very useful in phytoremediation purposes as *Portulaca oleracea*. This herbaceous plant could grow very well in several diverse habitats including neighboring channels of wastewater solutions. This plant has the capability in absorbing heavy metals, such as Cd, Cu, Zn, Hg, Pb and Se (Leguizamo et al. 2017).

There are several of *Portulaca* species in Indonesia, which are very possible to be employed as the agent of phytoremediation. But since along the edge of BSR were not found with several *Portulaca* species, therefore we decided only three species were chosen as candidate plants as phytoremediation agents. These three plants were *P. oleracea, P. glandiflora and P. pillosa* (Dalimartha 2009; Rynary 2012). The difference between them was recorded briefly below. The *P. oleracea* has been reported to be able to effectively removed EDC in the form of BPA (Bisphenol A) (Imai 2007). Meanwhile, *P. grandiflora* plants known as the green roof vegetation had shown its capability to translocate all metals examined, such as Al, Cu, Fe, and Zn (Vijayaraghavan 2016). Also, *P. grandiflora* has been proven to be able to decolorize sulfonated diazo dyes Navy Blue HE_2R ($NBHE_2R$) up to 98% in 40 hours (Khandare et al. 2011).

It is expected that those three *Portulaca* species would give a good response particularly in absorbing the EDC and could be promoted to be a plant agent of phytoremediation. By using those plants, therefore, the aims of this research project were to identify whether or not BSR subwatershed (Premulung, Pepe, Pucang Sawit, and Kalianyar) were contaminated by EDC and to examine the secondary metabolism of three *Portulaca* species in their saponin, tannin, and flavonoid.

MATERIALS AND METHODS

Sample collection

Water solutions of Bengawan Solo River (BSR) were collected from four different sites, namely 1. Premulung, 2. Pepe, 3. Pucang Sawit, 4. Kalianyar (Figure 1). Each research site was sampled 50 m in distance and the volume of water taken was 2 L from up and downstream from the river's outlet. There are 7 samples from BSR and two replication in each location. Meanwhile, *Portulaca* plants (mature) were collected from Ngringo Village, Karanganyar District, Indonesia and the edge of BSR.

Procedure of estrogenic identification

Sample preservation

The procedure of sample preservation in this experiment was adapted from Handayani (2005). To get a very clear water solution from the BSR, nitroacetat filter paper of 0,45 µm was used to get rid off of the solid waste product. The filtered water was then put in the sterile bottle and labeled and kept into the freezer to prevent any process of biodegradation during storage.

The solution of phenol standard, eluen, and HPLC test

The procedure of phenol standard, eluen and HPLC tests were adapted from Bellfroid (2005) and Imai (2007). Phenolic of the standard solution was made up by diluting 0.1 g of solid phenol standard into 1% of methanol solution, bringing the concentration into 100 ppm standard solution of phenol with concentration from 1-6 mg/L was made by diluting 0.1ml to 0.6 ml.

TLC (Thin Layer Chromatography) test

Sample preparation

The fifty grams of samples (leaves and steam) of *P. oleracea, P. grandiflora* and *P. pillosa* were picked up from the fresh material and then dried under the sun. The dried materials were then blended until becoming powder. This simplicia of each species was then used to extract the secondary metabolism, such as saponin, tannin, and flavonoid.

Extraction of secondary metabolites

The procedure of extracting secondary metabolism was adapted from Wahyuningsih (2008).

Five g of each simplicia from a single species was diluted into 100 ml of methanol. Afterward, the maceration was conducted for 48 hours and then evaporated under 50^0C until dried extract methanol was collected. This resulted in methanols were then dried and no smell was found. This solution was then kept in the porcelain petridish.

Detection of secondary metabolism

The presence of saponin compound in three species of *Portulaca*, i.e. *P. oleracea, P. glandiflora,* and *P. pillosa* were conducted using visible lights of 254 rays and 365 rays after steamed up with anisaldehyde sulfuric acid. Accordingly for the tannin observations were also done at the same wavelength of UV, i.e. 254 rays and 365 rays, respectively (B).

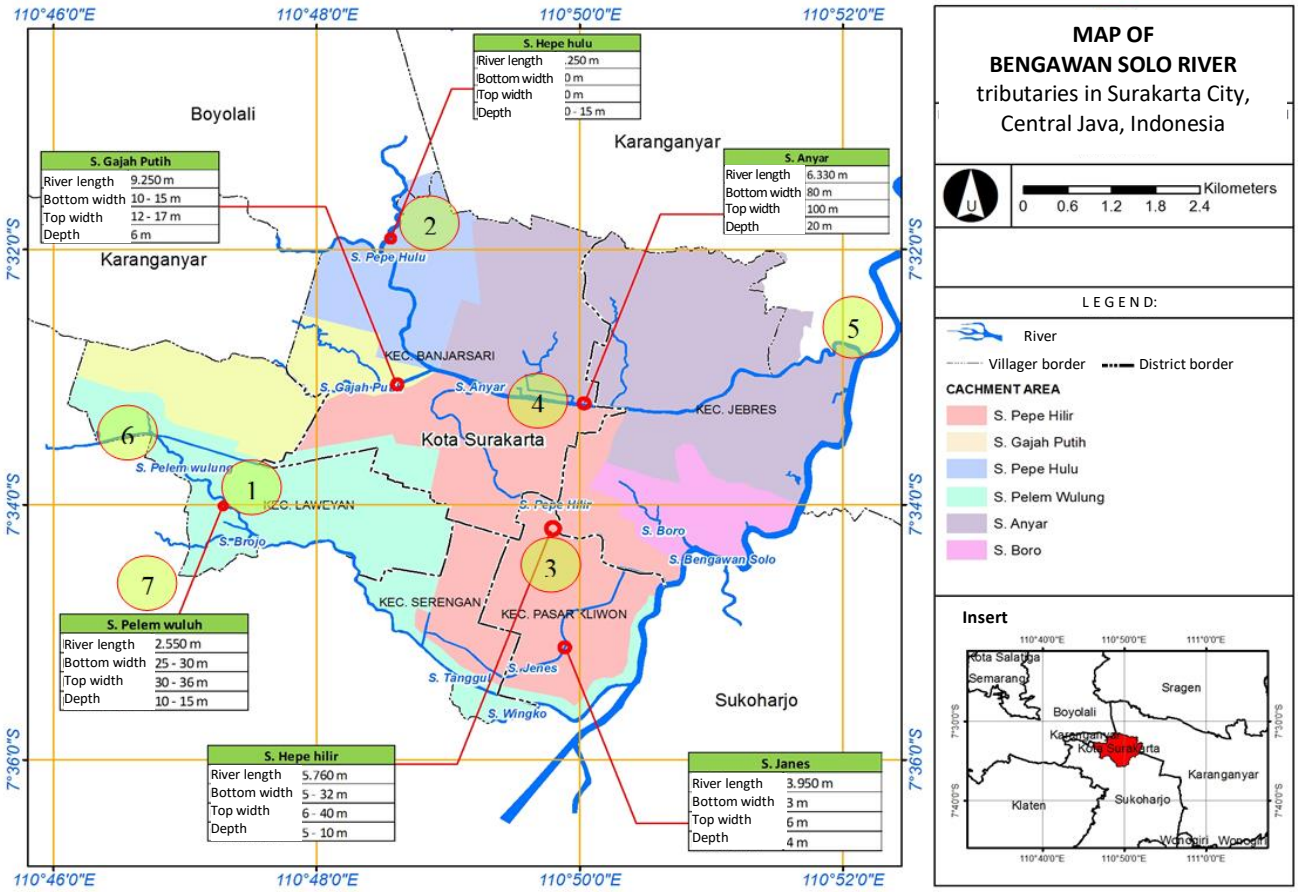

Figure 1. Map of Bengawan Solo River (BSR) in which four research sites used in collecting water for the estrogenic test. From 4 research sites; the only one sample location of Pucang Sawit River (1) was taken. Meanwhile, two samples of each river were collected; Pepe River A (2); Pepe River B (3); Kalianyar A (4); Kalianyar B (5); and the last two were Pelem Wulung A (6); Pelem Wulung B (7). Notes: 1-7 were the location for taken the samples.

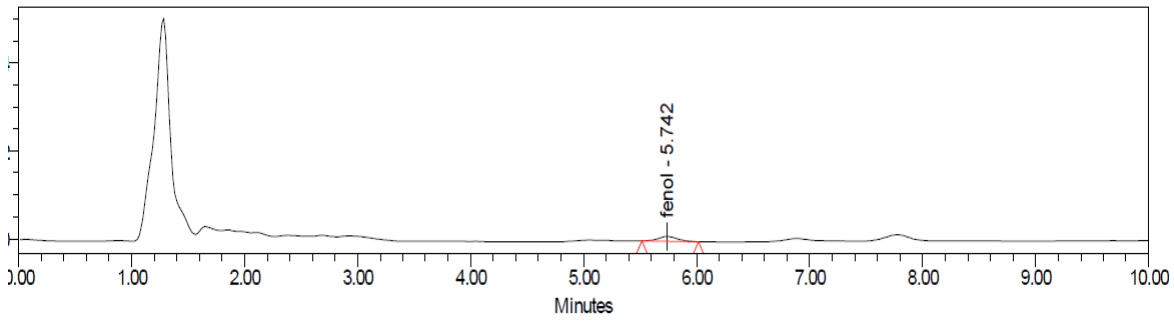

Figure 2. Chromatographic of the phenolic peak of water samples taken from four different rivers based on the HPLC test

Data analysis

The data obtained will be analyzed descriptively based on the TLC chromatogram and the HPLC curve standard.

RESULTS AND DISCUSSION

Phenolic compound in BSR

Phenolic test using HPLC for seven samples were taken at four different rivers (Fig.1) showed the only Pucang Sawit river particularly, the downstream appeared at the peak (5.742), it is indicated that this water has already

contaminated by the phenol (Figure 2). Meanwhile, the other 6 sample location sites of sampling did not. This occurrence may be one to the influence of environmental conditions such as temperature (Dewilda 2012). These results confirmed that in the area of Pucang Sawit river has been contaminated by the phenolic substance (0.013 ng/L), which is higher than the basic standard (0,001 ng/L) allowed by Indonesian government regulation of Republic of Indonesia No 82/2001.

This phenolic substance has been predicted as result of waste products of industrial activities, such as the home batik industry which was thrown away to the river. This waste product was considered to be very dangerous to the environmental conditions including human beings when this substance present in the human tissue in certain concentrations (Kulkarni and Kaware 2013). Phenol contaminants in the river could cause the unfordable environment, such as nasty smell or even cause the death of organisms in the water. It is also recorded that further severe effects of this phenolic contaminant could cause disrupting the function of human brains, lungs, rent or even blood circulation failure and died caused by breathing failure (Gami et al. 2014). Based on the HPLC test, phenol concentration of 0.013 ng/L was detected in the Pucang Sawit river. This contaminant substance resulted in no good smell and become an unfavorable condition for human beings who live surrounding the river. This condition was also shown by Utomo et al. (2017) in which the more concentration of phenol (0.0087-1.413 mg/L) has been detected particularly in the area of Solo-Sragen (part of BSR) in eastern Surakarta (Solo) City.

Phenol was one estrogenic compound that could disturb the endocrine hormone, which is usually called EDC (Wirasnita 2018). Another side effect of EDC on the water consumption to the female people was disturbing the activity of estrogen which is believed to be more feminine for people who drink this water. Accordingly, Beelfroid (1999) recorded that the above impact could prevent the growth and development of fish gonads and eventually would influence their fish sex behavior. It is also interested to note that this EDC contaminated drinks water could result in lowering down the total number of sperm production and maybe breast cancer (Anggraini 2017). The danger of this substance has also been recorded by Rattan (2017) particularly in the woman menstruation cycles which could be disturbed, and therefore could give effect in their fertility.

In recent years, the occurrence of shortening time for woman menstruation cycles may have been caused by the EDC contaminated substance (Binder et al. 2018). It is also predicted that the occurrence of early aging women may be due to this EDC (Wulansari 2016). It is interested in considering that whether or not that this water contaminated by EDC would directly or indirectly affect human health. Preliminary action such as this effort may need to be taken into action to minimize or reducing the danger of EDC in our environmental condition.

Based on the HPLC test, the sample from Pucang Sawit river has been detected phenolic compound. These may due to several environment influences. Dewilda (2016) argued that the location site and temperature have a symptom factor in detecting the characterized phenolic compound. The higher temperature would be eventually accelerated the evaporation of this substance and this phenomenon has been presented in this study. Phenol compound was detected in the first location of the river area of Pucang Sawit which close to the industrial area, at 08.00 am and 27^0C. Meanwhile for the other sample locations couldn't be detected and could be due to a bit late times (e.g 10.30 am) and the temperature has already reached 30-35^0C. These results were probably the reason why the phenol compound did not obtain.

***Portulaca* as phytoremediation**

Three species of *Portulaca,* i.e. *P oleracea, P. grandiflora,* and *P. pillosa* that grow at the certain edge of the Bengawan Solo river have been chosen as candidates of phytoremediation. The results of the TLC test for three species varied in their saponin, flavonoid, and tannin contents. There were no different patterns of Saponin content detected between plant samples of *P.oleraceae*. Meanwhile, the distinct spot was found for the sample of *P.grandifora*. This distinct spot was observed by the absence of spot number 2. In addition, no band variations were detected for both saponins in two other species. This occurrence was also found for the tannin and flavonoid content of *P.oleraceae* and *P. grandiflora* as well as *P. pillosa*. This unique appearance of saponin of *P.grandiflora* would be interested to test further in order to get a good plant candidate for phytoremediation purposes. These pictures could be seen in Figure 3.

The Rf values of *P. oleracea* 1 and 2 for their flavonoid were similar, meanwhile, the saponin content *P. oleracea* 2 was much higher (0.5). Within the flavonoid content, only *P. grandiflora* was detected to have a lower value of Rf as compared to the other 3 samples (0.92). Accordingly, the varied Rf values were also detected for their saponin content. *P pillosa* showed the highest value of Rf (0.78) while the others ranging from 0.21 to 0.5. It is interesting to note that for 4 samples taken from 3 species to have the same value of their tannin Rf (0.85).

The Study of using *P. oleracea* in degrading the EDC in form of Bisphenol (BPH) has conducted by Imai (2007). The study reported that the species could absorb EDC, such as octiphenol (OP), nonylphenol (NP); 2,4-dichlorophenol (2,4 DCP) and 17β-estradiol. This result confirms that the plant species is very promising to be applied in phytoremediation purposes for landfill leachates and waste products of industries contaminate by EDC. Based on the flavonoid and tannin test results of *P. oleracea,* the species will be quite promoting to be used for phytoremediation purposes. The ability of *P. oleracea* in absorbing the EDC has been considered very promoting due to its secondary metabolism content. The presence of secondary metabolisms, such as flavonoid, tannin, and saponin usually increases drastically under favorable environmental conditions. Under favorable conditions, these substances are usually produced in limited amounts and certain periods, as well as in very specific purposes of environmental conditions (Yang 2018).

Table 1. Result of phenolic identifications at four different sub rivers of BSR and resulted in seven (7) sample locations: In which the only Pucang Sawit river (1) showed phenolic contamination.

No.	Name of river sampling locations	Vial	Retention time	USP plate count	USP Tailing
1	Pucang Sawit	4	5,742	5735,33	1,0912
2	Pepe A	5	5,675	nd	nd
3	Pepe B	6	5,675	nd	nd
4	Kalianyar A	7	5,675	nd	nd
5	Kalianyar B	8	5,675	nd	nd
6	Pelem Wulung A	9	5,675	nd	nd
7	Pelem Wulung B	10	5,675	nd	nd
Mean			5,684		
Std Dev			0,025		

Notes: nd = not detected

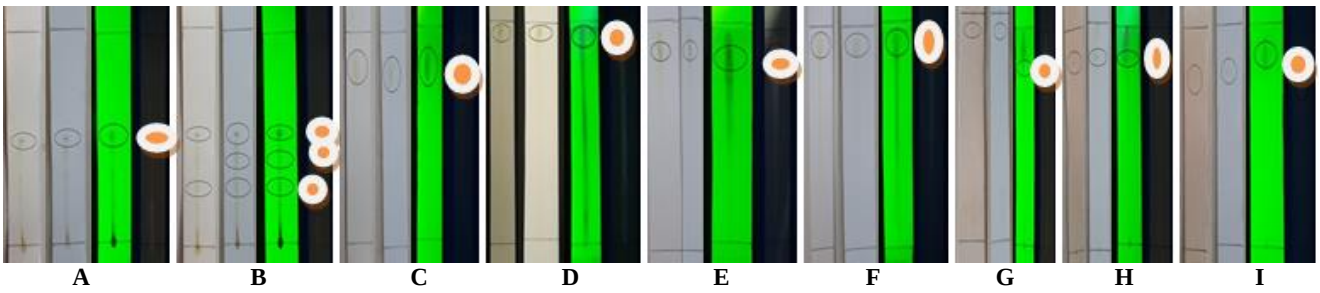

Figure 3. The results of TLC test for three species varied in their saponin, flavonoid, and tannin contents. Notes: A, s-o: saponin in *P. oleracea*, B. s-g: saponin in *P. grandiflora*, C. s-p: saponin in *P. pillosa*, D. f-p: flavonoid in *P. pillosa*, E. f-o: flavonoid in *P. oleracea*, F. f-g: flavonoid in *P. grandiflora*, G. t-o: tannin in *P. oleracea*,H. t-g: tannin in *P. grandiflora*, I. t-p: tannin in *P. pillosa*

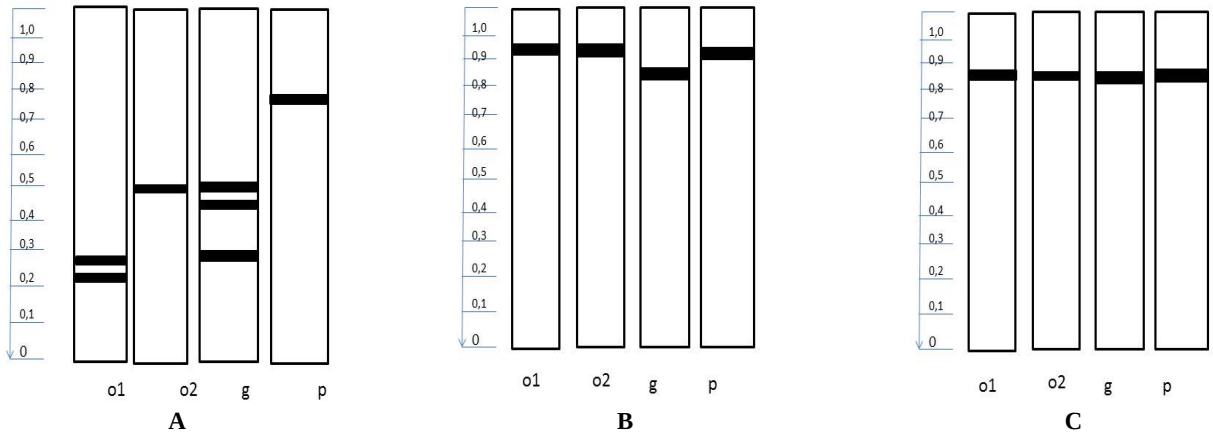

Figure 4. The Rf results of TLC test for three species varied in their saponin, flavonoid, and tannin contents. Notes : A. Saponin test *P. oleracea* (o1,o2) *P. grandiflora* (g) , *P. pillosa* (p); B. Flavonoid test *P. oleracea* (o1,o2) *P. grandiflora* (g), *P. pillosa* (p); C. Tannin test *P. oleracea* (o1,o2) *P. grandiflora* (g) , *P. pillosa* (p)

Another species of *Portulaca* which is very potential to be useful in reducing heavy metal water contamination is *P. grandiflora* (Vijayarashavan 2016). This species could be used in green roof vegetation, because it has the capability to translocating the metals, such as Al, Cn, and Zn (Vijayarashavan 2016). This species has already proven in rediscoloration of diazo sulfonation of Navi Blue HE2R (HBHE2R) until 98% within 40 hours. Decoloration of mixing textile industries was also shown by this species (Khandare et al. 2011) and this was supported by the TLC result of flavonoid and tannin of *P. grandiflora* and therefore this species has been considered very useful in reducing phenol contamination in the water.

Chandanshive et al. (2018) confirmed that *P. grandiflora* has capability in reducing color value and heavy metal contamination, such as Cd, As, Pb, and Cr.

This occurrence may be influenced by the activity of roots in improving specific activity of oxydo-reduction, such as lignin peroxidase, lactase, veratryl alcohol oxidase, tirosinase, and azo reductase during decoloration of textile activities in the soil. *P.oleraceae* could tolerate the pressure of Cr via high proline accumulation and improve more activities of peroxidase which resulted in a significant accumulation of Cr (150-190 mg/kg) (Kalee 2015). The capability of *P.oleracea* in reducing Cr in the environment would be effective in preventing the environment from the Cr contaminant. Using the presence of secondary metabolism in the plant tissues, it is very promising to use those species to be employed in phytoremediation purposes (Khandare et al. 2017).

Accordingly, the highest saponin content of *P. pillosa* would be attractive in inviting another organism to adapt to the environmental stress, preventing the UV light radiation or as the allelopathy substance (Yang 2018). It is assumed that if a higher secondary product of plants produced such as flavonoids and tannin, they may be able to absorb more the EDC than *P. oleracea*. Saponin has been considered to have an ability to improve plant adaptation under unfavorable environment. If the plants in which content more saponin may be able to survive, so this will be attractive in surrounding another organism.

In conclusion, based on the HPLC test it has appeared that water quality of branch Sub watershed-BSR has already been contaminated by EDC. The phenolic substance of EDC with a concentration of 0.013 ng/L was detected in the river of Pucang Sawit. Preliminary trial to overcome this problem by introducing three species of *Portulaca* as a candidate of phytoremediation agent resulting *P. pillosa* plant to have the highest saponin as compared to the other two species. This early result may be useful in considering *P. pillosa* to be applied shortly for phytoremediation purposes.

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