ANTIOXIDANT ACTIVITY AND TOXICITY EFFECT OF ELEVEN TYPES OF BARK EXTRACTS ACQUIRED FROM EUPHORBIACEAE

Saefudin[*1], Efrida Basri[2] and Agus Sukito[3]

[1]Biologi Research Center, Km.46, Jl. Raya Bogor, Cibinong, Bogor, West Java 16911, Indonesia
[2]Forest Product Research and Development Center, Jl. Gunung Batu 5, Bogor, West Java 16610, Indonesia
[3]Non-Timber Forest Product Technology Research & Development Institute, Jl. Dharma Bakti No. 7, West Lombok, Indonesia

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ANTIOXIDANT ACTIVITY AND TOXICITY EFFECT OF ELEVEN TYPES OF BARK EXTRACTS ACQUIRED FROM EUPHORBIACEAE. The use of natural antioxidants for medicinal purposes deserves thorough attention for their efficacy and possibly adverse toxicity. This paper studies the antioxidant actions and toxicity effects of bark extracts. The study focuses on eleven tree species of Euphorbiaceae family. Initially, bark samples from those trees were extracted using ethanol. The acquired extracts were examined for peroxide values with iodometric method. The bark extracts were chemically screened for possible antioxidant-compound contents, i.e. polyphenols, flavonoids, and saponins; and followed by oxidation-reduction test to assess the extract ability in vitro to scavenge free radicals in their standard sources, i.e. 2,2-diphenyl-1-picrylhydrazyl; altogether to determine qualitatively which species origin from bark extracts afforded the most potential as antioxidants. Toxicity test was performed on those bark extracts to assess their safety on living creatures, particularly humans as tried on shrimp larvae by counting their death, using the Brine Shrimp Lethality Test method. Results show that bark extracts of four plant species, i.e. *Acalypha hispida* Blume, *Bischofia javanica* Blume, *Glochidion arboreum* Blume and *Sapium baccatum* Roxb species afforded potentiality as antioxidants, because its peroxide value (POV) was lower than or somewhat above those of the positive control vitamin E (POV 89.45 µg/ml). However, bark extracts from *Euphorbia antiquorum* L, *Euphorbia hirta* L, and *Jatropha podagrica Hook* (i.e. LC_{50} : 238.85; 228.11 & 194.51 µg/ml) were highly toxic, because their LC_{50}'s value< 1000 µg/ml.

Keywords: Bark materials, ethanol extracts, peroxide value, antioxidant activity, toxicity

STUDI AKTIVITAS ANTIOKSIDAN DAN DAYA RACUN EKSTRAK KULIT 11 JENIS TANAMAN FAMILI EUPHORBIACEAE. Penggunaan bahan antioksidan alami untuk keperluan pengobatan perlu memperhatikan kemujaraban dan kemungkinan daya racunnya. Penelitian ini bertujuan mempelajari aktivitas antioksidan dan daya racun ekstrak kulit 11 jenis tanaman dari famili Euphorbiaceae. Awalnya, contoh kulit diekstraksi dengan etanol, dan hasilnya diperiksa bilangan peroksidanya dengan cara iodometri. Pemeriksaan fitokimia pada ekstrak kulit terhadap kemungkinan kandungan antioksidannya, yaitu polifenol, flavonoid, dan saponin; dilanjutkan dengan uji oksidasi-reduksi guna mencermati kemampuan ekstrak memangsa radikal bebas pada sumber baku (2,2-diphenyl-1-picrylhydrazyl/DPPH); guna menentukan ekstrak kulit mana yang paling berpotensi sebagai antioksidan. Eksrak kulit juga diuji toksisitasnya terhadap makluk hidup, khususnya manusia dicobakan pada jentik udang, menggunakan cara Brine Shrimp Lethality Test. Hasil penelitian menunjukkan ekstrak kulit dari empat jenis tanaman, yaitu Acalypha hispida *Blume,* Bischofia javanica *Blume,* Glochidion arboreum *Blume, dan* Sapium baccatum *Roxb berpotensi sebagai antioksidan karena nilai peroksidanya (NP) lebih rendah atau sedikit di atas nilai peroksida kontrol positif vitamine E (NP 89,45 µg/ml). Akan tetapi ekstrak kulit dari* Euphorbia antiquorum *L,* Euphorbia hirta *L dan* Jatropha podagrica *memiliki daya racun yang tinggi (masing-masing: 238,85; 228,11 dan 194,51 µg/ml) karena nilai* LC_{50} *< 1000 µg/ml.*

Kata kunci: Bahan kulit, ekstrak etanol, bilangan peroksida, aktivitas antioksidan, toksisitas

[*] Corresponding author: saefudinkahfi@yahoo.com

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I. INTRODUCTION

The use of natural antioxidant is obtained usually through the extraction from particular portions of plants (e.g. bark, leaves, stems and twigs) and has been developed extensively in current medicines. Depending on the content and kind of their natural compounds in such plant extracts, they have proven their efficacious ability in treating or curing various diseases and reducing free radicals. Three popular compounds that could serve as significant sources of natural antioxidants are flavonoids, polyphenols, and saponins (Akbarirad, Ardabili, Kazemeini, & Khaneghah, 2016; Harvard T.H. Chan School of Public Health, 2016). Those antioxidant compounds are particularly found in plant bark (Lukmandaru, Vembrianto, & Gazidy, 2012; Saefudin, Marusin, & Chairul, 2013). Accordingly, testing the content and toxicity of flavonoids, polyphenols and saponins as antioxidant compounds in particular plant species is necessary to look into the efficacy of their portions (e.g. bark, leaves, stems, and twigs) as medicinal plants.

Euphorbiaceae ranks the fourth among the five largest families of woody vascular plants in the Indo-Malesia. Based on research, it was found that there are about 148 species of vascular plants that belong to Euphorbiaceae family, which exhibit potentiality as traditional herbal medicine (Djarwaningsih, 2011). Several studies have reported that particular plant species of Euphorbiaceae were already used as an expectorant, asthma, laxative, kidney ailments, and as a diuretic medicine; and the essential oils extracted from the flowers of *Acalypha hispida* plant (also belonging to this family) were capable of antioxidant actions (Onocha, Oloyede, & Afolabi, 2011).

Pharmacological studies have been conducted on several plant species of Euphorbiaceae; and the results showed their empirical antioxidant effect. Those species included *Acalypha indica*, *Aleurites moluccana*, *Euphorbia antiquorum*, *Phyllanthus niruri* and *Sauropus androgynus*. Further, disclosure of antioxidant potentiality is associated with bioactive compounds contained in the plant extracts. One of the effective indicators to determine efficacy of antioxidant compounds is by examining its peroxide value (POV), which demonstrates or indicates the compound ability to react with free radicals; and accordingly those compounds are able to inhibit or slow down the oxidation process of special fatty matters (particularly their unsaturated components) in biomass bodies. On the other hand, possible toxicity test that might be inflicted by plant materials that actually exert beneficial antioxidant action is very important to be carefully considered and thoroughly watched as a medicinal herb to secure the living creatures, particularly humans. Toxic effects of plant extracts in vitro cultures can also be used to examine the ability of extract nature to inhibit the proliferation of cancer cells, e.g. Hella cell cancer (Saefudin, Syarif, & Chairul, 2014). This study is aimed to identify the potentiality of antioxidant activity and toxicity of the eleven bark extract types, each obtained from eleven plant species of Euphorbiaceae. Their antioxidant activity was evaluated by the extract's ability to prevent or inhibit lipid peroxidation exerted by hydrogen peroxide (POH), and ability to reduce (scavenge) free radical at 2.2-diphenyl-1-pikrilhidrasil (DPPH). Meanwhile, toxicity testing was intended as safety precaution of the extracts in their use as herbal drugs, which implemented the so-called Brine Shrimp Lethality Test (BSLT) methods.

II. MATERIAL AND METHOD

A. Materials

The materials were bark portions cut from the stem of 11 tree species, approximately at their breast height. Those 11 species originated from West Java, which are comprised of *Acalypha hispida* Blume, *Baccaurea lanceolata* (Miq.) Müll. Arg, *Bischofia javanica* Blum, *Codiaeun variegatum* (L.) A.Juss, *Croton paniculatus* Lam., *Euphorbia antiquorum* L., *Euphorbia hirta* L., *Jatropha podagrica* Hook, *Glochidion arborescens* Blume, *Macaranga tanarius* (L.) Müll.Arg, and *Sapium baccatum* Roxb.

In their original locations, those trees grew as natural forests, with their unknown ages and varying diameters.

The chemicals used included ethanol, acetic acid, chloroform and sodium thiosulfate (Na_2SO_3) 0.1 N, concentrated hydrochloric acid (HCl), sodium chloride (NaCl), dimethyl sulfoxide (DMSO), and potassium iodate (KIO_3). Other chemicals used were distilled water and starch, 1% solution. The materials for extracts toxicity tests were larvae of shrimp (*Artemia salina* Leach). The tools used were: hammermill, rotary evaporator, freeze dryer, erlenmeyer flasks, and vials (sterile container).

B. Methods

1. Extraction

All of those bark samples of the 11 tree species origin were dried, shaped into powder, and filtered to obtain a coarse-sized powder (8-mesh size). Further, a total of 200 grams of powder per bark type (species origin) was weighed and macerated (extracted) with 70% ethanol solvent for 24 hours. Subsequently, the solvent that already contained bark extract was filtered; and then the obtained filtrate was concentrated with a rotary evaporator until the filtrate volume was reduced to 100 ml, and afterwards dried in a freeze dryer to obtain a dry bark extract.

2. Peroxide test of bark extracts and their ability for scavenging DPPH's free radicals

The peroxide value (POV) test referred to Williams as adopted by Saefudin and Basri, (2016). A total of 5 g dry bark extract was prepared, put into the 100 ml erlenmeyer tube (flask) and then it was added by 30 ml of acetic acid-chloroform (3:2). The tube (flask) is still being shaken, 0.5 ml of a saturated solution of KI and 30 ml of distilled water was added, then titrated using 0.1 N $Na_2S_2O_3$, and it was added again 0.5 ml of 1% starch solution (as indicator), which immediately caused the mixed solution to turn blue colored. The titration was continued until the blue color of solution disappeared and became colorless; and the volume of $Na_2S_2O_3$ titrant was recorded. Likewise, similar procedures were performed on the standard antioxidant agent, i.e. vitamin E (alpha-tocopherols) as a control. Determination of the peroxide value (POV) for both the alleged antioxidants (in bark extracts) and vitamin E used the formula:

POV (mg) = S x N x 1/1000 g sample .....(1)

where, S = volume of sodium thiosulfate (ml) solution added as titrant; N= normality of sodium thiosulfate solution.

The experiment that dealt with peroxide test was arranged in a completely randomized design (CRD) with single factor. The factor (treatment) was the 11 tree species origins of bark extract samples, whereby three replications were used for each species. The response (observed parameter) was the POV values. If there was a difference in POV among those 11 species, then further assessment proceeded with the Duncan's multiple range tests at 5% level.

Further, the ability of the bark extract in reducing (scavenging) free radicals at DPPH in this study was compared with the POV for vitamin E similarly to cope with the DPPH's free radicals. This is because vitamin E has been used as a natural antioxidant and serves as reductor in the oxidation-reduction process; and consequently as scavenger for DPPH's free radicals. Further, vitamin E typifies as fat-soluble antioxidants. Vitamin E in its roles (and possibly other antioxidant agents with almost similar chemical structures) acts as a radical scavenger (including also the DPPH's free radicals) by delivering its hydrogen (H) atoms to such free radicals (Grossi, Di Lecce, Arru, Toschi, & Ricco, 2015; Evans & Lawrenson, 2017). Activities of how high and convincing the potentiality of the bark extracts was as antioxidant to reduce the DPPH's free radical were assessed with the aid of spectrophotometer device at particular wavelengths (520 nm), by calculating the reduction percentage of DPPH (Q) (Molyneux, 2004; Sharma & Bhat, 2009). Value 0 = no DPPH's free radical scavenging,

while the value of 1 (100%) = total or high damping (scavenging) of free radical. Likewise, the reduction percentage of DPPH by the vitamin E was also assessed for comparison (control). The Q formula (including also the Q value for vitamin E) is as follows:

$$Q = 100 (A0-A1) / A0 \quad \text{.....(2)}$$

where: A0= Initial absorbance (DPPH solution), at ± 520 nm wavelength; A1 = Absorbance of the DPPHsolution (at ± 520 nm as well) after the addition of bark-extract with certain and similar concentrations, among different bark-extract.

3. Phytochemical screening

Phytochemical screening was conducted on bark extracts of the 11 tree species origin to determine qualitatively if particular components possibly contained in the extracts, which were allegedly capable of antioxidant activity (e.g. scavenging the free radicals). Such allegation focused on 3 (three) prevalent bioactive antioxidants, which comprised polyphenols, flavonoids, and saponins. The method used in this screening test referred to Guevera (2005).

a. Polyphenol

Ten miligram of bark extract of each tree species origin was inserted into a test tube and then dissolved into 10 ml of hot water. While stirring, 5 drops of 10% NaCl was added into the test tube and it was shaken until the solution became homogeneous. The solution was divided by pouring into two other test tubes. The first served as a positive control tube; and to the second tube was added three drops of reagent (10% $FeCl_3$ solution). Bark extract was regarded as positively containing polyphenols as hydrolyzed compounds, if the extract solution in the second tube turned into blue or dark-blue color. Conversely, if the solution in the second tubes changed to turquoise color, this indicated the presence of polyphenols as the condensed compounds.

b. Flavonoids

Ten miligram of bark extract of each species origin was inserted into a test tube and then hexane solvent was added to extract its coloring pigment exhaustively. The pigment-containing hexane solution was then dried to remove (evaporate) residual hexane. Five mililitre of 80% ethanol was further added into the test tube, and vigorously shaken until it became homogenous. The homogenous solution was subsequently divided into two test tubes. Half mililitre of concentrated HCl and 3-4 drops of magnesium metal granules were added into the first tube. If the color of the solution in the first tube changed and became red, it would be positive of flavonoid presence. Further, the second tube was added with 0.5 ml of concentrated HCl, and then heated over the water bath for 15 minutes. After one hour, if the color of the solution in the tube changed to intense red or violet color, it indicates that the extract contained leuco-anthocyanin.

c. Saponins

Ten miligram of the bark extracts of any tree species origin in a test tube was reconstituted (mixed) with 5 ml of 80% ethanol and then it was added with 5 ml of distilled water. The mixture was shaken vigorously and left to stand for 30 minutes until it became foamy. If the foam height exceeded 3 cm from the upper surface boundary, it will indicate that the bark extract contain saponins.

4. Toxicity test

The toxicity test in this matter used the method called the Brine Shrimp Lethality Test (BSLT) (Meyer et al., 1982). Materials for this test were similarly the bark extracts obtained from the assayed extraction when testing the antioxidant activity. In this regard, the obtained bark extracts of any species origin were dissolved in ethanol, and then the resulting ethanol extract solution was made varying with their staged concentrations progressively, i.e. 0 µg/ml (without bark extract, just ethanol

solvent as a control), 10 μg/ml, 100 μg/ml, 1000 μg/ml, and 2000 μg/ml, using the solvent of an artificial seawater (3.8 grams of crude salt, which was not iodized, dissolved in 1 liter of distilled water) and DMSO (dimethyl sulfoxide) solution (with concentration of 20 ml per 1 liter of distilled water). Each dosage (concentration) of the solution that incorporated artificial seawater and DMSO solvents was made in triplicate with 5 ml volume; and then inserted into the 20-ml vial (sterile container) on which had been marked the volume up to 10 ml. Further, 50 mg of *Artenia salina* shrimp cysts was inserted into another container that already contained the artificial seawater. Half of the container was left open for exposure to light illumination. After 3 days, the shrimp cysts inside the container would become adult (mature) larvae and were ready for the test.

In the next step, a total of 10 mature shrimp larvae were taken from their container; and then inserted into the vial already given 10-ml volume mark, which was previously contained bark extract solution in the mixture of artificial seawater and DMSO solvents in various concentration (dosages), i.e. 0–2000 μg/ml. The larvae inside the vial were left for 24 hours. Observation was conducted by counting the number of the dead larvae. The observation results were expressed as the value of LC_{50}, which signified at what figure was the concentration of the bark extract sample solution that caused the death of as much as 50% of shrimp larvae as of their original number (total) after 24 hour incubation period. The LC_{50} values became the parameters whether the alleged active substances (compounds) were regarded as toxic, less toxic, or not toxic. If the LC_{50} value <1000 μg/ml, then the compound (i.e. bark extract solution in ethanol solvent at the interpolated concentration) could be described as toxic. The smaller the LC_{50} value the more toxic would be the compound; and on the contrary for the greater values. In this regard, the convincing LC_{50} value of ethanol extract solution was figured out using the Probit Finney method (McLaughlin, Rogers, & Anderson, 1998).

The experiment associated with the toxicity test, similar to the previous peroxide test, was arranged in CRD with single factor/treatment. The treatment was also for 11 tree species origins of bark extract solution, with three replications per species origin. The response/ observed parameters were the LC_{50} values. Further assessment proceeded with the 5% level Duncan's multiple range test should a difference occur in the LC_{50}'s among those 11 species.

III. RESULTS AND DISCUSSION

A. Potentiality of Antioxidant

Data in Table 1 revealed as many as 11 types (tree species origin) of ethanolic bark extracts as each originated from 11 tree plant species of Euphorbiaceae family, and their varying peroxide values (POV) that ranged about 81.43-191.56 μg/ml. Out of those 11 types, there were 4 types of bark extracts that exhibited POV lower than or somewhat above those of the positive control (vitamin E; 89.45 μg/ml), which comprised, i.e. *A. hispida* (81.43 μg/ml), *B. javanica* (90.56 μg/ml), *G. arborescens* (91.10 μg/ml), and *S. baccatum* (91.35 μg/ml). This indication demonstrated the ability of those four types of ethanolic bark extracts in inhibiting the oxidation process, which were more effective than or almost as an effective as vitamin E. Meanwhile, the remaining 7 types, which consisted of *M. tanarius* (POV 191.56 μg/ml), *E. antiquorum* (POV 183.40 μg/ml), *J. padagrica* (POV 181.80 μg/ml), *C. paniculatus* (POV 125.15 μg/ml), *E. hirta* (POV 105.78 μg/ml), and *C. variegatum* (POV 104.85 μg/ml) also exerted their ability to inhibit the oxidation process but they were not as effective as vitamin E. It is causes by the fact that peroxide values were far greater than of for vitamin E.

Ethanol bark extracts which exhibited POV lower than the stipulated criteria (100 μg/ml) exerted high potentiality in decreasing or scavenging free radicals released by 2.2-diphenyl-1-1 picrylhydrazyl (DPPH); and

Table 1. Peroxide values (POV) of 11 types of ethanol bark extracts originating from 11 plant species of Euphorbiaceae

No.	Species origin (Scientific name)	Species origin (Local name)	POV (µg/ml)
1.	*Acalypha hispida* Blume	Ekor kucing	81.43a
2.	*Baccaurea lanceolata* (Miq.) Müll.Arg	Lempaung	111.04c
3.	*Bischofia javanica* Blume	Gadog	90.56b
4.	*Codiaeun variegatum* (L.) A.Juss.	Puring	104.85bc
5.	*Croton paniculatus* Lam.	Tutup putih	125.15d
6.	*Euphorbia antiquorum* L	Patikan kebo	183.40e
7.	*Euphorbia hirta* L.	Patikan kebo	105.78bc
8.	*Jatropha podagrica* Hook	Jarak	181.80e
9.	*Glochidion arborescens* Blume	Mareme	91.10b
10.	*Macaranga tanarius(L.)* Müll.Arg	Mara	191.56e
11.	*Sapium baccatum* Roxb.	Ludai	91.35b
Positive control		(Vit. E)	89.45b

Remarks: Mean values followed by the same letter in horizontal direction means they are not significantly different, a < b < c < d (based on Duncan's multiple range test, at 5% level)

conversely the reverse was true. One particular bark-extract type from its corresponding plant species origin (*A. hispida*) afforded the lowest POV at 81.43 µg/ml (Table 1), and therefore regarded as the most potential for antioxidant. Further, bark extract from *B. javanica* with POV at 90.56 µg/ml was judged as the second most potential for antioxidant (herbal medicine). There are several factors that could cause such different POV values with varying types of bark extracts, among others the presence of double or triple carbon bonds of particular organic compounds (usually lipid/fat matters) inside the extracts; and the extract content of antioxidant agents. The double or triple bonds are chemically unstable and therefore prone to chemical changes due to heat, light, and oxidation actions, forming the so-called unstable peroxide compounds (Grossi et al., 2015). On the other hand, such changes (especially oxidation) could be prevented or hindered due to the presence of presumably antioxidants in the bark extracts themselves, such as polyphenols, flavonoids, and saponines (Adebiyi & Abatan, 2013).

Nowadays, local people in North Sulawesi utilize bark of *B. javanica* species as ingredients for the drug in curing disease, lumbago (low back pain), and energy enhancer. However, the POV values as described above just indicate the potentiality of a particular substance (including in this regards bark extracts) to be used as antioxidant. This is because such POV values only measure the extent to which a substance has undergone the so-called auto-oxidation, which are merely free-radical reaction involving oxygen that leads to chemical changes on the substance, e.g. decomposition and ageing. (Grossi et al., 2015). Consequently, in order to ascertain the role of the allegedly antioxidant substance, results of POV examination should be continued with the so-called DPPH (2,2-diphenyl-1-picrylhydrazyl) test. The DPPH is typically an organic substance composed of stable free-radical molecules. Accordingly, the DPPH is used most commonly in the laboratory assay test to monitor chemical reactions that involved radicals (Sharma & Bhat, 2009).

Regarding the bark-extract efficacy as free-radical scavenging or reduction, it was indicated by the particular absorbance intensity of those four bark extract types (Table 2). Absorbance measurement of the bark extract samples of 11 species origin (types) was performed after 14-day storage, which used a spectrophotometer device. In using the spectrophotometer, it was

Table 2. Absorbance and ability of scavenging (reducing) the DPPH free radical by four types of ethanol bark extracts

Species origin of bark extracts	Absorbance in 1000 ppm	Reduction/ scavenging ability for lowering free radicals, %**)
Acalypha hispida Blume *)	3.86	92.03
Bischofia javanica Blume *)	3.07	90.89
Glochidion arborescens Blume *)	4.29	92.56
Sapium baccatum Roxb. *)	4.36	94.25
Positive control (Vit. E)	3.17	93.54
Negative control (aqueous ethanol liquid; no bark extracts)	0.82	

Remarks: *) at similar initial particular concentrations of bark extracts; **) high reduction> 50%; fair reduction>20-50%; low reduction <20%

repeated in triplicate in order to obtain more appropriate or convincing absorbance values as well as scavenging-ability figures for any of those four bark-extract types. The obtained values of absorbance varied from 3.07 to 4.36 (Table 2). Meanwhile, the absorbance values for positive control (vitamin E) and negative control (aqueous liquid, no bark extracts) were consecutively 3.17 and 0.82. These results suggested that those four types of bark extracts provided satisfactory absorbance values, as indication for their efficacious antioxidant (Table 2), which were mostly far above or differed significantly from those of the positive control/vitamin E (3.17) and of the negative control (0.82). Meanwhile, the absorbance value of B. javanica's bark extract (3.07) was slightly below, but it was still regarded as comparable to that of positive control /vitamin E (3.17).

There were three types of bark extracts with high absorbance values i.e. *A. hispida*, *G. arborescens* and *S. baccatum* exceeding that of vitamin E (3.17), while the value of *A. hispida*'s bark was the lowest (3.86) (Table 2). Further, it was revealed that the greater the absorbance value, the greater would be the scavenging ability figures of the alleged anti-oxidant materials, i.e. bark extracts and vitamin E (Table 2). Consequently, results of absorbance and scavenging ability test following the DPPH's assay strongly (Table 2) confirmed the POV's antioxidant potentiality of those four bark extracts (POV values were lower than or close to the vitamin E's POV) (Table 1), whereby the indicated potentiality of *S. baccatum* Roxb's bark extracts was the highest, followed in decreasing order by *G. arborescens* Blume, *A. hispida* Blume , and *B. javanica* Blume bark extracts, respectively as the lowest (Table 2). However, it strongly suggested that the activity of bioactive components in *S. baccatum* Roxb bark extracts was more complete and its antioxidant activity afforded the highest (94.25%) value, which was above the capability of vitamin E (93.54%) in reducing (scavenging) the DPPH's free radical (Table 2). The potentiality of antioxidant components of *A. hispida* plants besides being found in their bark portion (the second lowest scavenging ability, 92.03%) also existed in their leaves. The vitamin E and the alleged antioxidants in bark extracts that could perform the H-atom delivery to the DPPH molecules lead to the situation that the DPPH's free radicals were as if preyed or scavenged by those antioxidants (Evans & Lawrenson, 2017).

All those four bark extract types (i.e. *A. hispida*, *B. javanica*, *G. arborescens*, and *S. baccatum*) exhibited high antioxidant potentiality, with their peroxide value (POV) <100 μg/ml at the test using 1000-ppm concentration (Table 1). Besides, in reality those extracts entirely afforded high activity in reducing/scavenging

the DPPH's free radical, as their scavenging ability was far above 50%, in the range of 90.89 –94.25% (Table 2). Therefore, the activity of those extracts in scavenging the DPPH's free radical was categorized as very good. In this study, ethanol extraction of *G. arborescens*'s bark reached 92.56% in reducing the DPPH's free radical (Table 2), which was still higher than the methanol extraction performed by Marusin, Saefudin and Chairul (2013) who achieved only 87.06%. Meanwhile, the activity of reducing the DPPH's free radicals by ethanol extract of *B. javanica* (90.89%) and *S. baccatum*'s bark (94.25%) did not differ much from that of *G. arborescens*'s bark (92.56%).

Further, the high absorbance values (or high reduction/scavenging ability for DPPH's free radicals) (Table 2) were not always followed by the low POV values for each of those four bark extract types (Table 1) and the reverse was so as well. This situation could be attributed to different antioxidant agents in any of the bark extract types, either qualitatively or quantitatively, e.g. polyphenols, flavonoids, and saponines (Table 3). Moreover, the POV values as described before related mostly to the extent of auto-oxidation that have occurred to bark extracts' compounds with possible free radical releases (Ali, Wahid, Khatune, & Islam, 2015), while the more confirmed DPPH's assay focused more on the scavenging of DPPH's free radicals by the alleged antioxidant agents in those extracts (polyphenols, flavonoids, and saponines) as well as by vitamin E (Sharma & Bhat, 2009). Despite uncertain relation in results between the POV test and DPPH's free radical assay, it could be asserted convincingly that those four bark extract types with lower POV values (or somewhat higher) than the values for vitamin E (Table 1) could in fact afford the reduction or scavenging of DPPH's free radicals as much as 90.89–92.45%, comparable spectacularly with those of vitamin E as well (Table 2).

Still further, when referred to the results of phytochemical screening test (Table 3), the antioxidant activities exerted by bark extract types of *A. hispida* and *S. baccatum* was regarded as quite strong (Table 2), if compared to the types of *B. javanica* and *G. arborescens*. Bioactive screening results strongly indicated qualitatively that *A. hispida* and *S. baccatum*'s bark extracts contained the most polyphenol compounds (+++). These results suggested the potentiality of such compounds in the ethanol extract in *A. hispida* and *S. baccatum* barks other than for antioxidant uses, which could be used as herbal medicine. Polyphenols typify as compounds which have the basic structure of phenol units with more than one hydroxyl (OH) groups, whereby the OH groups are attached directly to an aromatic hydrocarbon ring (Dhianawaty & Ruslin, 2015). Polyphenols yielded by particular plant species exerted antioxidant properties and is effective in preventing dangerous diseases such as cancer (Miryanti, Sapei, Budiono, & Indra, 2011), heart attacks, and blood vessel disease (Rodella & Favero, 2013).

When compared to the apparent polyphenol presence (content) in the positive control (vitamin E), the apparent contents in bark extracts from *A. hispida* Blume and *S. baccatum* Roxb. were similar; but still greater than the apparent polyphenol contents in *B. javanica* Blume and *G. arborescens* Blume bark extracts (Table 3). This situation was almost commensurate with the more confirmed DPPH's assay results, whereby the ability of *A. hispida* Blume and *S. baccatum* Roxb bark extracts as the alleged antioxidants to scavenge DPH's free radicals as much as 92.03% and 94.25%, respectively was conveniently comparable to those of vitamin E (93.54%) (Table 3). From these phenomena, it could be judged that the polyphenols in particular types of bark extracts took substantial roles in reducing the DPPH's free radicals.

A. hispida's bark extract was also indicated qualitatively to contain the highest flavonoid (+++) compounds (Table 3). Flavonoids also belong to polyphenols which can inflict positive effects on human health as free-radical scavenger, since they can donate H atoms (reducing agent) to the free radicals

thereby stabilizing those radicals, hence not inducing oxidation (Puspitasari, Wulansari, Widyaningsih, Maligan, & Nugrahini, 2016), and removing toxic metals from the human body (Kumar, Mishra, & Pandey, 2013; Kumar & Pandey, 2013). Flavonoid compounds easily change, due to the influence of oxidation, light, and chemical agents, thereby decreasing the function of its active ingredient and solubility. Stabilizing and improving the solubility of flavonoids can be done by converting them into glycosides form.

When compared to the apparent flavonoid content in the positive control (vitamin E), the apparent contents in *A. hispida* Blume bark extracts were similar; and exhibited the greatest value (Table 3), followed in decreasing order by *S. baccatum* Roxb. and *G. arborescens* Blume bark extracts (both as the second apparent greatest), and ultimately by *B. javanica* Blume bark extracts (as the apparent lowest). This situation, however, was rather inconsistent with the DPPH's assay results, whereby the ability of *S. baccatum* Roxb. bark extract to scavenge DPPH's free radicals was the greatest, while the ability of *A. hispida* Blume bark extracts to do so was the second lowest (Table 2). This occurrence strongly indicates that although it is able to serve as antioxidant, however, the role of flavonoids in bark extracts to scavenge the DPPH's free radicals was not so pronounced compared to the role of polyphenols.

Saponin as bioactive components was also qualitatively present in bark extracts with its content categorized indicatively as low (+) to moderate (++) compared to that with positive control/vitamin E (+++) (Table 3). In this study, saponin was supposedly found only slightly (+) in *A. hispida* bark extract. Saponin belongs to a class of complex natural compounds in the form of glycoside. This antioxidant can decrease LDL (low-density lipoprotein) cholesterol in the blood, inhibit the growth of colon cancer and is able to neutralize blood sugar by stimulating insulin secretion from the pancreas (Vinarova et al., 2015).

The apparent saponine contents in *B. javanica* Blume, *G. arborescens* Blume, and *S. baccatum* Roxb bark extracts, respectively seemed similar to each other; while the apparent content in *A. hispida* Blume bark extracts was the lowest (Table 3). Further, all the apparent saponine contents in those four bark extract types were lower than the apparent content in the positive control (vitamin E). These phenomena were notably inconsistent with the results of DPPH's assay test (Table 2), whereby the ability of *S. baccatum* Roxb bark extracts as the alleged antioxidant to reduce (scavenge) the DPPH's free radicals exhibited the greatest (94,25%) value, which was even greater than the ability of vitamin E (93.54%). This occurring situation strongly suggests that, almost similar to the case of flavonoid' alleged antioxidant, the role of saponine' antioxidants to scavenge the DPPH's free radicals was less efficacious than the role of polyphenols. Further, judging from all the overall phenomena (Tables 2 and 3), which convincingly indicates that the role of polyphenols as the alleged antioxidant to

Table 3. Bioactive compounds indicatively present (identified) and yielded from the screening test on four types of bark extracts with their corresponding species origin

Species origin for bark extracts	Bioactive compounds		
	Polypenol	Flavonoids	Saponin
Acalypha hispida Blume	+++	+++	+
Bischofia javanica Blume	++	+	++
Glochidion arborescens Blume	++	++	++
Sapium baccatum Roxb.	+++	++	++
Positive control (Vit. E)	+++	+++	+++

Remarks: +++ apparently enormous/intensive/strong reaction occurred; ++ apparently fair/moderate reaction; + apparently slight/weak reaction

scavenge the DPPH's free radicals was the strongest (Table 3), compared to the other alleged antioxidants (flavonoids and saponine), with its achievement closer to the role of positive control (vitamin E).

To sum up, those four types of ethanol bark extracts with species origin (i.e. *A. hispida*, *B. javanica*, *G. arborescens* and *S. baccatum*), following the peroxide test, which exhibited the POV below or almost similar to that of vitamin E (Table 1); and following oxidation-reduction test for the DPPH's free radical scavenging ability, which ranged about 90.89–94.25% (Table 2), after their chemical screening test for bioactive components (Table 3), were in part strengthened regarding their efficacy as the alleged antioxidants.

B. Toxicity Tests and Possible Effects

Results of toxicity test on wood bark extracts of 11 plant species origin (Table 4) revealed the varying activity associated with their LC_{50} values, beginning from being very toxic (LC_{50} 170.86–347.87 µg/ml), moderately toxic (LC_{50} 424.59–659.12 µg/ml), until weakly toxic (LC_{50} 659.78–932.82 µg/ml) (Table 4). In accordance with the criteria stipulated by Meyer et al. (1982), bark extracts originated from 5 species were regarded as toxic, because their LC_{50}'s value < 1000 µg/ml. Bark extracts of three particular plant species origin, which comprised *Euphorbia antiquorum* L (LC_{50} 238.85 µg/ml), *Euphorbia hirta* L (LC_{50} 228.11 µg/ml), and *Jatropha podagrica* Hook (LC50 194.51 µg/ml) belonged to those species which were the most toxic, since they inflicted the most sensitive (deadly/lethal) effect on *A. salina* shrimp larvae. This is because their low LC_{50} values lay in the range regarded as very toxic (LC_{50} 170.86-347.87 µg/ml), as described above; and further resulted in the death of numerous larvaes. Meanwhile, bark extracts from *Bischofia javanica* Blume (LC_{50} 508.31) and *Glochidion arboreum* Blume (LC_{50} 522.38) were regarded as moderately toxic. Ultimately, bark extracts from six other species were judged as not toxic or safe, and therefore could be used as secure or harmless traditional medicine for the community in the village vicinity.

The use of *Jatropha podagrica* Hook (castor oil-bearing) seeds by local community is as traditional medicine particularly to cure ringworm diseases, former injury, and giving birth (baby delivery). The secondary metabolite compounds which are toxic to the living creatures typify as alkaloids that exist in the extracts of the nine species origin for castor-oil bearing seeds (Table 4). Others bioactive compounds, particularly in the extract of Jatropha podagrica bark comprised among others fraxidin, fraxetin, scoparone, 3-acetylaleuritolic acid, β-sitosterol and sitosterone (Rumzhum et al., 2012). Utilization of those compounds in traditional remedies is to cure dysentery, bronchitis, breast inflammation, typhus, kidney and milk gland irritation.

Bark extracts of 9 out of 11 plant species origin were tested for their toxicity (Table 4), were categorized as being poisonous (toxic), due to their LC_{50} values < 1000 µg/ml (Meyer et al., 1982). Accordingly, almost all those extracts could exhibit potentiality as natural herbal drug. Consequently, precautionary measures should be thoroughly taken in their uses, particularly when determining the concentration or dosages of those herbal drugs. Accordingly, bark extracts with high toxicity should be used in low dosages; and conversely for those with low toxicity.

Wood bark extracts originating from B. javanica (LC_{50} 508.31) and *G. arboreum* (LC_{50} 522.38 µg/ml were regarded as fairly or moderately toxic according to the Meyer (1982)'s criteria, because their LC_{50} values were in the range of 424.59-659.12 µg/ml. Meanwhile, bark extracts from *Euphorbia antiquorum* L. (LC_{50} 238.85 µg/ml), *Euphorbia hirta* L. (LC_{50} 228.11 µg/ml), and *Jatropha podagrica* Hook (LC_{50} 194.51 µg/ml) were judged as the most toxic, because they exhibited very strong deadly activities according to those criteria (their LC_{50} values were far below the criteria's LC_{50} values).

With respect to the four particular bark extracts each originated from four tree

Tabel 4. LC_{50} values for ethanol bark extracts of eleven plant species origin

No.	Species origin	Local name	LC_{50}
1.	*Acalypha hispida* Blume	Ekorkucing	1113.87
2.	*Baccaurea lanceolata* (Miq.) Müll. Arg	Lempaung	902.20
3.	*Bischofia javanica* Blume	Gadog	508.31^{++}
4.	*Codiaeun variegatum* (L.) A. Juss.	Puring	804.56
5.	*Croton paniculatus* Lam.	Tutup putih	902.50
6.	*Euphorbia antiquorum* L.	Patikan kebo	238.85^{+++}
7.	*Euphorbia hirta* L.	Patikan kebo	228.11^{+++}
8.	*Jatropha podagrica* Hook	Jarak	194.51^{+++}
9.	*Glochidion arboreum* Blume	Mareme	522.38^{++}
10.	*Macaranga tanarius*(L.) Müll. Arg	Mara	985.46
11.	*Sapium baccatum* Roxb.	Ludai	1080.37

Remarks: +++ Enormously/extraordinarily toxic); ++ Fairly/moderately toxic; +++ Highly/very toxic; LC_{50} = lethal concentration of the alleged toxic substance (i.e. bark extracts in this regards) that could kill as many 50% of the individual organisms of their original number

species, which have been judged as efficacious antioxidant agents (Table 3), *Acalypha hispida* and *S. baccatum* Roxb. bark extracts were regarded as safe or not toxic to living creatures, as already tested against *A. salina* shrimp larvae with their LC_{50} values of 1113.87 µg/ml and 1080.37 µg/ml, respectively (both > 1000 µg/ml) (Table 4); and therefore the bark extracts from those two species origin could be regarded as harmless in their use for antioxidants (A.O.T., Orekova, & Yakubu, 2012; Adebiyi & Abatan, 2013). Meanwhile, *B. javanica* Blume and *G. arborescens* Blume bark extracts, judged also as efficacious antioxidants (Table 3), were considered as moderately toxic (LC_{50} values 508.31 µg/ml and 522.31 µg/ml, both still in the range of 424.59-659.12 µg/ml). Accordingly, special precautionary measures on those two bark types should be thoroughly taken for their uses as antioxidants.

Scrutinizing Table 3, *A. hispida* Blume and *S. baccatum* Roxb. bark extracts apparently exhibited greater presence (content) of polyphenol as well as flavonoid compounds than *B. javanica* Blume and *G. arborescens* Blume bark etracts. Meanwhile, saponine presence/contents in *B. javanica* Blume, *G. arborescens* Blume, and *S. baccatum* Roxb. bark extracts, respectively seemed to be similar to each other; and the saponine content in *A. hispida* Blume bark extract was apparently the lowest. However, judging from the toxicity test results (Table 4), it turned out that *A. hispida* Blume and *S. baccatum* Roxb. bark extracts were regarded as harmless or non-toxic, because their LC_{50} values (1113.87 and 1080.37 µg/ml, respectively) were greater than 1000 µg/ml; while *B. javanica* Blume and *G. arborescens* Blume bark extracts afforded moderate toxicity with their LC values (508.31 and 522.38 µg/ml, respectively) lower than 1000 µg/ml. These phenomena accordingly led to the strong indication that the varying (different) toxicity behaviors (actions) among those four types of bark extracts, as tested against *A. salina* shrimp larvae, were not related with their presence (content) of those three alleged antioxidants (i.e. polyphenols, flavonoids, and saponine). Instead, such toxicity difference could be due to the varying contents/presence of presumably specific toxic ethanol-soluble compounds (other than those three alleged antioxidants) in the extracts, such as quercetin, taxifolin, lignans, stilbenes, glycosides, alkaloids, and phlobaphenes (Sjostrom, 2013; Mota et al., 2017).

The death of *A. salina* shrimp larvae inflicted by particular bark extracts became a beneficial parameter to indicate their content of active

compounds which were toxic. The toxicity rate of a compound could be assessed from its LC_{50} value, using the so-called probit-log concentration graph. If the LC_{50} values < 1000 µg/ml, then their corresponding compounds were judged as toxic. In other words, the smaller the LC_{50} values, the more toxic would be the compounds. Accordingly, bark extracts originating from *A. hispida* and *S. baccatum* species with their LC_{50} values greater than 1000 µg/ml were regarded as safe or not toxic to be used as herbal drugs (Table 4). Meanwhile, bark extracts from other species such as *Baccaurea lanceolata* (Miq.) Müll. Arg, *Codiaeun variegatum* (L.) A. Juss., *Codiaeun variegatum* (L.) A. Juss, *Croton paniculatus* Lam, *Macaranga tanarius* (L.) Müll. Arg and *Sapium baccatum* Roxb still could be used safely as traditional medicines, particularly for curing ringworm diseases, former injury, and baby delivery, although they were regarded as less effective. That was based on the content of polyphenols, flavonoids, and saponins in the bark extracts as examined in this research. Therefore, the belief adopted by the community as the herbal remedy was made possible due to the activity of other chemical contents than those three compounds. Based on field observation, the utilization of bark portion from those plant species by the community around the forests in Banten, Lampung, and Bengkulu was caused by their ease to obtain. In addition, those plant species were also growing in the vicinity of their house as ornament or decorative plants.

IV. CONCLUSION

Wood bark extracts originating from four out of eleven plant species that belonged to Euphorbiaceae family, i.e. *Acalypha hispida* Blume, *Bischofia javanica* Blume, *Glochidion arboreum* Blume, and *Sapium baccatum* Roxb., exhibited their potentiality as antioxidant sources. Results, phytochemical screening strongly suggested that there was a strong association between high free radical scavenging and amount of antioxidant compounds, particularly polyphenols in wood bark.

Toxity test revealed that bark extracts from *B. javanica* (LC_{50} 508.31) and *G. arboreum* (LC_{50} 522.38) species were able to inflict fair or moderate toxic effect. Meanwhile, bark extracts from *Euphorbia antiquorum* L. (LC_{50} 238.85 µg/ml), *Euphorbia hirta* L. (LC_{50} 228.11 µg/ml), and *Jatropha podagrica* Hook (LC_{50} 194.51 µg/ml) could deliver the most toxic effect or afford very strong toxicity actions.

Utilization of bark extracts from six other plant species origins, i.e. *Baccaurea lanceolata* (Miq.) Müll. Arg, *Codiaeun variegatum* (L.) A. Juss., *Codiaeun variegatum* (L.) A. Juss, *Croton paniculatus* Lam, *Macaranga tanarius*(L.) Müll. Arg, and *Sapium baccatum* Roxb., was for traditional medicines to cure dysentery, bronchitis, breast irritation, kidney troubles, and milk gland inflammation. However, their use as herbal drugs was less effective, viewed from their content of polyphenols, flavonoids, and saponins. Accordingly, community belief in bark uses as the herbal remedy became possible due to the activity of other chemical contents than those three compounds.

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