

Short Communication: Identification and evaluation of bioactivity in-forest plants used for medicinal purposes by the Kutai community of East Kalimantan, Indonesia

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Manuscript received: 5 September 2017. Revision accepted: 9 January 2018.

Abstract. Zarta AR, Ariyani F, Suwinarti W, Kusuma IW, Arung ET. 2018. Short Communication: Identification and evaluation of bioactivity in forest plants used for medicinal purposes by the Kutai community of East Kalimantan, Indonesia. *Biodiversitas* 19: 253-259. The Indonesian forest is one of the most species-rich ecosystems in the world. Within such forests are plant species with secondary metabolites that have novel molecular structure and diverse biological activity with excellent potential to be used medicinally in prevention and cure of various diseases afflicting humans. Plant materials often contain various forms of antioxidants. Phenolic compounds found in plants have many biological effects. Flavonoids and other phenolics play a protective role against metabolic damage caused by disease and environmental stressors. The communities of Kutai Kartanegara in East Kalimantan Indonesia are representative of many traditional peoples who have evolved ways of treating human ailment and disease by use of specific plants sourced from their forests. The purpose of the research described in this paper was to identify significant medicinal plant species used by the Kutai ethnic community and to prepare extracts from these plants, mainly from the leaves, and to evaluate the extracts for bioactivity; namely by general identification of secondary metabolites, and by estimation of their antioxidant activity, toxicity, and anti-bacterial activity. Samples of ten plant species, used medicinally by the Kutai community, were extracted using ethanol solvent. Assay of antioxidant activity was carried out by the spectrophotometric method using DPPH (1,1-diphenyl-2-picrylhydrazyl radical) as the control. The degree of toxicity of the extracts was determined by the BSLT (Brine Shrimp Lethality Test) while anti-bacterial activity was evaluated using an *in vitro* assay of growth inhibition of cultures of the bacterium *Escherichia coli*. The result showed that nine of the plant species had strong antioxidant activity (IC₅₀); extracts of two of the species were very toxic, while one other was toxic; and at least eight of the species had extracts that exhibited anti-bacterial activity. The phytochemical compounds identified in several of the ten species included flavonoids, tannins, saponins, steroids, triterpenoids, and alkaloids.

Keywords: Traditional medicinal plants; antioxidants; secondary metabolites; toxicity

INTRODUCTION

Forests in Indonesia are among the richest ecosystems in the world. The Indonesian forests contain more than 400 species of trees that are highly valued economically, as well as more than 25,000 species of flowering plants (Bioresercher 2013). From these plants, secondary metabolites with biological activity and novel molecular structure have potential to be developed as cures for various diseases that afflict humankind.

Knowledge about human diseases, both ancient and modern, is increasing year by year, but it is recognized that many existing drugs are often less effective in combating disease organisms that have developed resistance to the drugs or in combination newly identified disease organisms with different modes of pathogenicity. Therefore, the search for new types of drugs is an on-going activity,

especially amongst the natural resources of tropical forests that have been only partially explored.

The development of herbal medicines and drugs from plant species is often limited by lack of scientific information about the medical efficacy of the plant species. The recognition of this fact has stimulated research into use of plants for medicinal purposes by traditional communities that have had long intimate connections with forest ecosystems in the environments they inhabit. The necessity of scientific proof of the efficacy of such traditional medicinal herbs is a prerequisite to their being developed by the pharmacological industry in Indonesia (Sampurno 2003). Plant materials contain various kinds of biologically active compounds such as antioxidants. Phenolic compounds found in plants have many biological effects. Flavonoids and other phenolics play roles in protecting plants against various metabolic threats and many have the potential to similarly protect humans against damage

caused by disease (Kähkönen et al. 1999; Mammadov et al. 2011).

Traditional medicines used by the people of Kutai Kartanegara in East Kalimantan, Indonesia, are very diverse. These medications and treatments can be classified into two types; namely curative drugs, and traditional medicinal treatments of pain and external and internal medical conditions. Apart from their general knowledge of traditional medicines, the Kutai ethnic community place faith in the specific knowledge and skills of traditional healers and herbalists (Achmad 1990).

Based on a survey in 2016, several types of forest plants were identified that traditionally have been used by the Kutai ethnic community as medicinal ingredients in treating particular diseases. Traditional treatments have often evolved in the past from experimenting with preparation of extracts from plant species recognized to have interesting properties. If the results of the experiments were good, then treatment with the extracts was continued until healing occurred. If the preliminary experiments did not produce good outcomes, then the treatment was stopped, and other plant species were tried instead. As a result traditional medicines prepared from many types of plants have been tested over time as useful for treating particular diseases. The use of plants in disease treatment has sometimes also extended to the combination of several types of plant materials.

In our study, we focused on a number of plants species for which the traditional knowledge as to their efficacy in treatment of medical conditions had strong local support among the Kutai community. We sought to test whether scientific evidence into the bioactive ingredients contained in extracts from these plant species would give credence to the traditional beliefs about the benefits of their use in medical treatment. The goal of the research was to identify the bioactivity of extracts from these forest plants used medicinally by the Kutai; in particular, to identify the general presence of secondary metabolite compounds; and to evaluate the antioxidant activity of the extracts, as well as their toxicity and anti-bacterial effectiveness.

MATERIALS AND METHODS

Reagents

Ethanol; acetone; dimethyl sulfoxide (DMSO); 1,1-diphenyl-2-picrylhydrazyl radical (DPPH); ascorbic acid; reactants for Dragendorff's test, Liebermann-Burchard test, and Molisch test; potassium dichromate ($K_2Cr_2O_7$); Nutrient Broth medium (NB); and the antibiotic Chloramphenicol.

Plant material and extraction

Plant materials in the form of leaves and roots from ten plant species with recognized phytochemical interest were collected in November 2016 in the vicinity of Desa Sebulu Modern, Kutai Kartanegara regency, in East Kalimantan province, Indonesia (Table 1). The plant species were identified in the Research Institute for Natural Resource Conservation Technology, at Samboja, in Kutai

Kartanegara Regency. The plant materials were dried at room temperature and ground into a powder (Arunkumar and Muthuselvam 2009). The dried materials from the ten species – *Callicarpa longifolia* Lam (100 g), *Tetracera* sp. (100 g), *Bridelia glauca* Blume. (100 g), *Tetrastigma* sp. (100 g), *Leea indica* (Burm. f.) Merr. (100 g), *Urena lobata* L. (60 g), *Clinacanthus nutans* (Burm. f) Lindau (100 g), *Allophylus cobbe* (L.) Raeusch (100 g), *Alstonia iwahigensis* Elmer (100 g), *Hippobroma longiflora* (L.) g. Don (30 g) – were extracted in ethanol at room temperature for 48 hours. Then the extracts were filtered, and the concentrates were prepared by using a rotary vacuum evaporator at a temperature of 30-40°C. The amount of concentrate obtained from the ten plant species varied from 1.70 g to 8.66 g.

Phytochemical analysis

Phytochemical analyses were performed for detection of alkaloids, flavonoids, and tannins (Kokate 2001), as well as for steroids, saponins, and triterpenoids (Harbone 1984).

Analysis of antioxidant activity

The investigation of antioxidant activity was carried out using the method of Arung et al. (2008). A spectrophotometer was used at temperature room (25°C) and 514 NM wavelength. DPPH solution (1,1-diphenyl-1-picrylhydrazyl radical), as well as ascorbic acid (Vitamin C), were used as positive controls. The concentration of the sample extract needed to achieve inhibition of 50% was expressed as the IC₅₀ value for the extract. There were three replicate analyses per extract and the results were averaged. Determination of the antioxidant activity of the extracts using the DPPH method was carried out according to Jun et al. (2003).

Toxicity testing

Toxicity testing of the plant extracts used the Brine Shrimp Lethality Test (BSLT) described by Meyer et al. (1982). The BSLT method is widely used to obtain an approximate measure of bio-activity of plant materials with suspected medicinal application. These methods are easy to perform, are inexpensive, fast, and able to be used with small amounts of plant extract (Meyer et al. 1982). The BSLT method is also widely used for screening new potential anticancer compounds derived from plants. The results of toxicity tests using this method have demonstrated a correlation with cytotoxic anti-cancer activity. LC₅₀ is defined as the concentration of a compound that is expected to kill 50% of a test population within a given time interval (Boyd 2005). Determination of the level of toxicity used the aquatic toxicity criteria defined by Wagner et al. (1993).

Anti-bacterial testing

The antimicrobial test was performed by the diffusion method described by Cappucino and Sherman (2001), with some modification. In this test, 20 ml of Nutrient Broth medium (NB) was poured into a sterilized petri dish. After that, the media was hardened and flattened using a cotton

swab, in an aseptic state (using laminar flow). The media were allowed to dry for approximately 30 minutes.

Hole wells were made using a cork borer applied to the media. The wells contained 20 µL with different amounts of extract: 25 µg / well, 50 µg / well, 100 µg / well and 200 µg / well. Acetone was used as a negative control and Chloramphenicol as a positive control. Bacterial incubation was performed for 24 hours, and then the inhibition zone was measured around the wells in each petri dish.

RESULTS AND DISCUSSION

Traditional medicine of the Kutai ethnic community

Among the Kutai ethnic community of Desa Sebulu Modern, in Kutai Kartanegara Regency of East Kalimantan, there is traditional knowledge of medical treatments based on medicinal herbs growing in their forest vegetation. This knowledge has been inherited from their ancestors and has been preserved from generation to generation. However, not everyone is expert in the types of forest plants that can be used as herbal medicines. Only certain people have this kind of ability and such persons are usually acknowledged for their expertise in concocting medicinal cures.

Based on information and discussions with such traditional healers in the Kutai ethnic communities, each generation attempts to develop the knowledge gained from their ancestors. This includes knowledge about the forest vegetation in general, but also of methods used in concocting medicines by way of mashing, dissolving and boiling specific herbs. Such methods sometimes make people reluctant to consume herbal medicines because the aromas are often pungent and the tastes bitter.

The types of medical conditions that are treated with herbal medicines from the forests range from mild ailments such as flu, colds, cough, headaches, and stomach pain through to serious disease conditions such as cancer,

strokes, heart attacks, hypertension, constriction of blood vessels, stomach injuries, kidney stones, and others.

The results of our identification of ten plant species utilized by the Kutai community in Desa Sebulu Modern for such traditional medical purposes are summarized in Table 1.

Phytochemical content

Phytochemical testing of plant materials is used to identify secondary metabolite compounds. Such compounds while not a requirement for normal body function, are often found on evaluation to have positive effects on human health and can play an active role in prevention and treatment of disease. The specific results of our phytochemical analyses, listed in Table 2, indicate that amongst the plant extracts prepared from the ten species, secondary metabolite compounds included flavonoids, tannins, saponins, steroids, triterpenoids, and alkaloids. The presence of these phytochemical compounds in the plant materials suggests the potential medicinal value of their extracts in the prevention and/or cure of specific diseases.

Based on the results of our research, the ten plant species differed somewhat in the presence or absence of specific secondary metabolites, as has been reported in other studies of this kind (Ayoola et al. 2008).

Flavonoids were found in all the medicinal plant species tested, a result similar to the findings of Khodadadi (2015). In general, flavonoids can function as antioxidants which inhibit possible metabolic damage by free radicals (Hanani et al. 2005). All these potential medicinal plants showed antioxidant activity (see the next Section). The presence of flavonoids and tannins in plants has been found to play a role in binding free radicals. Flavonoids and tannins are phenolic compounds, and phenolics, in general, are a group of compounds that act as primary antioxidants in binding free radicals in plant metabolic pathways (Evans 2009).

Table 1. Plants species and their parts used by the Kutai ethnic community as traditional cures in treating particular medical ailments

Scientific name	Family	Local name	Traditional utilization	Plant part used
<i>Callicarpa longifolia</i> Lam.	Lamiaceae	Rehau	Bloody diarrhea; vaginal bleeding, cleanses the blood; diabetes	Leaf
<i>Tetracera</i> sp.	Dilleniaceae	Kayu/akar amblas	Dysentery; bleeding	Leaf
<i>Bridelia glauca</i> Blume.	Phyllanthaceae	Kayu tadah/rukam	Hypertension; bleeding; relapse (physical weakness)	Leaf
<i>Tetrastigma</i> sp.	Vitaceae	Akar kempis	Dissolving fat	Spreading root
<i>Leea indica</i> (Burm.f.) Merr.	Vitaceae	Mali (berduri)	Hypertension; vomiting blood; fever; malaria; lumbago	Leaf
<i>Urena lobata</i> L.	Malvaceae	Pulutan	Rheumatism; wounds	Leaf
<i>Clinacanthus nutans</i> (Burm.f) Lindau	Acanthaceae	Akar belau	Diabetes; malaria; fever; jaundice	Leaf
<i>Allophylus cobbe</i> (L.) Raeusch.	Sapindaceae	Ambau	Diarrhea; diabetes	Leaf
<i>Alstonia iwahigensis</i> Elmer	Apocynaceae	Pelai	Blood stream; diabetes; blood pressure; male vitality; lumbago	Leaf
<i>Hippobroma longiflora</i> (L.) G. Don	Campanulaceae	Tapak leman	Kidney stones; stamina/ vitality	Leaf

Table 2. Types of secondary metabolite compounds identified in the phytochemical analysis of extracts from ten medicinal herb species

Local names of the plants	Chemical contents					
	Flavonoid	Tannins	Saponins	Steroid	Triterpenoid	Alkaloid
<i>Callicarpa longifolia</i> Lam.	+	+	-	+	-	-
<i>Tetracera</i> sp.	+	+	+	-	+	+
<i>Bridelia glauca</i> Blume.	+	+	-	+	-	-
<i>Tetrastigma</i> sp.	+	+	-	+	-	-
<i>Leea indica</i> (Burm.f.) Merr.	+	+	-	+	-	+
<i>Urena lobata</i> L.	+	+	-	+	-	-
<i>Clinacanthus nutans</i> (Burm.f.) Lindau	+	-	+	+	-	+
<i>Allophylus cobbe</i> (L.) Raeusch.	+	+	-	+	-	+
<i>Alstonia iwahigensis</i> Elmer	+	-	-	+	-	-
<i>Hippobroma longiflora</i> (L.) G. Don	+	+	+	+	-	+

Free radicals are a by-product of metabolic processes in biological cells and tissues. They are molecules or atoms that are chemically unstable because of abnormal arrays of electrons. The existence of these chemically unstable free radicals can affect the ability of enzymes to maintain cell functions. To take one example in humans, skin deterioration and aging arise from damage to the collagen and elastin brought about by free radicals generated by environmental and metabolic impacts over time. Free radicals can be generated by factors external to the body such as by food containing various preservatives, color additives, and unsaturated fatty acids; by pesticides; by ultraviolet radiation; and by cigarette smoke, among others (Potterat 1997). The value of natural antioxidants in inhibiting the activity of free radicals generated by such factors is thus an important area of current medical research.

In biological systems, flavonoids have antioxidant activity; inhibiting free radicals, and in some cases having anti-allergic effects with reduction in inflammation and platelet aggregation. There are also reports of antimicrobial effects, and inhibition of ulcers, tumors, and hepatotoxicity (Okwu and Ndu 2006). In general, phytochemical analysis of plants with suspected medicinal benefits is a fruitful area of research for identifying physiologically active compounds with the ability to protect the human body from the effects of various kinds of metabolic damage arising from both internal and external factors (Igwenyi et al. 2011).

Antioxidant activity

The search for compounds with high antioxidant levels among plants recognized by traditional ethnic communities as being medically efficacious is more likely to produce

positive research outcomes than would be a random exploration of forest plants unguided by such traditional knowledge. Pisoschi and Negulescu (2011) emphasize the importance of focusing on the geographical distribution of specific plant populations in the search for biologically relevant antioxidants.

In our investigation of the antioxidant activity of extracts from the ten medicinal plants from Desa Sebelu Modern, the free radical scavenging effect of the fractions at different concentrations was measured by DPHH assay using a spectrophotometric determination of absorbance at a wavelength of 517 nm (Jun et al. 2003; Cefarelli et al. 2006; Ebrahimzadeh et al. 2009; Saeed et al. 2012). The percentage inhibitory effect of different concentrations of the extracts was expressed relative to the controls as follows:

$$\text{Inhibition\%} = 100 \times (\text{DPHH control absorbance} - \text{sample absorbance}) / (\text{control absorbance})$$

The IC₅₀ value (the concentration required to obtain a 50% inhibition) was employed as the parameter to express the relative antioxidant capacity of the different plant extracts. The antioxidant effectiveness of a plant extract is judged to be strong if it has a value of IC₅₀ <50 ppm. Estimates of the IC₅₀ values, shown in Table 3, were obtained by linear regression analysis of the trend in% inhibition in response to increasing concentration of the extracts.

The results of the study indicate that the antioxidant inhibition of free radicals by the tested samples increased with increasing concentration of the relevant extract in the samples. The results showed that in general, the plant extracts had significant antioxidant activity with IC₅₀ values of less than 50 ppm. An exception to this was the extract obtained from *Alstonia iwahigensis* Elmer which had a very low antioxidant activity with an IC₅₀ value of 140.48 ppm (Table 3). The results suggest that except *A. iwahigensis*, the plant species used by the Kutai community for medicinal purposes have significant antioxidant activity, a property which may be part of the explanation for the reputed curative efficacy of plant extracts from these species.

Toxicity

The toxicity testing of the plant samples aimed at finding out whether the samples contain a toxin or not. The brine shrimp *Artemia nauplii* has been suggested to be used as a model species in some evaluations of the pharmacological activity of ecotoxins and large complex compounds (McLaughlin et al. 1993; Dvorak et al. 2010). In our toxicity testing, we used the related shrimp species *Artemia salina*, Linnaeus. Initial tests were carried out with all ten extracts to see if at concentration of 0.1% (1000 µg/mL) the extracts would kill the shrimp larvae. In this preliminary test, three of the sample extracts at that concentration resulted in a shrimp larvae mortality of 50% or more (see Table 4).

Table 3. Antioxidant content and calculated IC50 values of extracts from ten plant species used medicinally by the Kutai community

Sample	Conc. (ppm)	Inhibition (%)	IC50
<i>Callicarpa longifolia</i> Lam.	50.00	61.35	39.06
	25.00	35.34	
	12.50	21.35	
	6.125	12.18	
	3.125	7.07	
<i>Tetracera</i> sp.	1.562	3.46	2.30
	12.50	75.77	
	6.125	62.25	
	3.125	50.99	
	1.562	47.89	
<i>Bridelia glauca</i> Blume.	12.50	78.09	5.88
	6.125	54.73	
	3.125	35.31	
	1.562	30.90	
<i>Tetrastigma</i> sp.	12.50	84.62	7.79
	6.125	35.94	
	3.125	17.02	
	1.562	7.44	
<i>Leea indica</i> (Burm.f.) Merr.	12.50	58.78	10.48
	6.125	31.00	
	3.125	19.35	
	1.562	9.86	
<i>Urena lobata</i> L.	12.50	70.61	8.53
	6.125	34.05	
	3.125	24.19	
	1.562	22.22	
<i>Clinacanthus nutans</i> (Burm.f.) Lindau	12.50	12.03	27.26
	6.125	6.49	
	3.125	4.27	
	1.562	1.74	
<i>Allophylus cobbe</i> (L.) Raeusch.	25.00	86.82	5.54
	12.50	84.96	
	6.125	63.72	
	3.125	23.41	
<i>Alstonia iwahigensis</i> Elmer	50.00	19.01	140.48
	25.00	11.34	
	12.50	7.03	
	6.125	5.11	
	3.125	3.51	
<i>Hippobroma longiflora</i> (L.) G. Don	1.562	1.92	8.08
	12.50	68.39	
	6.125	45.33	
	3.125	34.39	
	1.562	8.75	

Table 4. Evaluation of the toxicity of extracts from ten medicinal plant species used by the Kutai Ethnic community – mortality of *Artemia* larvae exposed to concentrations of 0.1% (1000 µg/mL) of the extracts

Sample	Mortality (%)	Assessment
<i>Callicarpa longifolia</i> Lam.	37	Not toxic
<i>Tetracera</i> sp.	50	Toxic
<i>Bridelia glauca</i> Blume.	50	Toxic
<i>Tetrastigma</i> sp.	7	Not toxic
<i>Leea indica</i> (Burm.f.) Merr.	3	Not toxic
<i>Urena lobata</i> L.	7	Not toxic
<i>Clinacanthus nutans</i> (Burm.f.) Lindau	3	Not toxic
<i>Allophylus cobbe</i> (L.) Raeusch.	7	Not toxic
<i>Alstonia iwahigensis</i> Elmer	3	Not toxic
<i>Hippobroma longiflora</i> (L.) G. Don	60	Toxic

According to Meyer et al. (1982), a plant extract is considered to have toxic activity if it is able to kill more than 50% *Artemia* larvae at a concentration of 1000 µg/ml. Therefore we went on to test in more detail the toxic activity of three plant extracts. We evaluated their toxic effect at lower concentrations i.e. at 0.05%, 0.025%, 0.01%, and at 0.001%. The results are displayed in Table 5.

The higher the concentrations of the extracts in solution, the higher was the percentage mortality in the test larvae. The trends in percent increase in mortality (%) in response to increase in extract concentration (µg/mL) were analyzed by liner regression to obtain estimates of the LC50 for the three sample extracts. The results of the calculation of LC50 values indicate that samples extracted from *Tetracera* sp. and *Bridelia glauca* were not toxic, while sample *Hippobroma longifolia* was assessed to be toxic. These findings are of significance because if concoctions extracted from these three species are being used in traditional medicines by the Kutai communities, then it needs to be determined whether it is safe for human consumption irrespective of potential benefits such as antioxidant activity of their phytochemical compounds. No complaints have so far been voiced against use of these materials as ingredients in the Kutai traditional medicine. Based on this fact, it is suspected that the toxic effects of the extracts on *Artemia* shrimps possibility do not extend to harmful effects on humans.

Anti-bacterial activity

Many traditional medicines derived from plants are identified as having anti-microbial activity. One standard method for preliminary testing of such antibiotic activity is to determine whether extracts of the medicinal plant can inhibit the growth of the ubiquitous, potentially pathogenic, bacterium, *Escherichia coli*. *E. coli* is an enteric bacterium of the family *Enterobacteriaceae*. It is pathogenic because it can cause infection in humans and animals. A bacteriologist, Theodor Escherich, identified *E. coli* in pigs suffering from enteritis; inflammation of the intestine that can cause abdominal pain, nausea, vomiting, and diarrhea both in humans and animals. *E. coli* can live in different environments: soil, water, plants, animals, and humans (Berg 2004; Bhunia 2008; Manning 2010). Enteric bacteria can survive in the human digestive tract including structures of the oral cavity, esophagus, stomach, intestine, rectum, and anus. *E. coli* can live in both aerobic and anaerobic environments. Therefore, the bacterium is categorized as a facultative anaerobic (Manning 2010).

We tested the anti-bacterial effects of the plants extracts from the ten medicinal species of the Kutai community. The extracts were tested at four different concentrations for their efficacy in inhibiting the growth of *E. coli* bacteria cultured on a Nutrient Broth medium in petri dishes. The results of the assay are summarized in Table 6.

The *E. coli* bacteria is pathogenic because it can cause infection in humans and animals. A bacteriologist, Theodor Escherich, identified *E. coli* in pigs who suffer from enteritis. Enteritis is an inflammation of the intestine that can cause abdominal pain, nausea, vomiting, and diarrhea both in humans and animals. *E. coli* is a bacteria that can

live in different environments. These bacteria can be found in soil, water, plants, animals, and humans (Berg 2004; Bhunia 2008; Manning 2010).

Escherichia coli bacteria is a family of Enterobacteriaceae which belongs to enteric bacteria. Enteric bacteria are bacteria that can survive in the digestive tract including structures of the oral cavity, esophagus, stomach, intestine, rectum, and anal. *E. coli* can live as aerobic bacteria and anaerobic bacteria. Therefore, *E. coli* is categorized as a facultative anaerobic (Manning 2010).

The extent of the bacterial inhibition zone for each of the ten extracts was greater at higher concentrations of the extract. A classification of strong inhibitory activity is given if the width of the zone of inhibition exceeds 6 mm, a classification of moderate inhibitory activity if the zone is 3-6 mm, and of weak inhibitory activity if the zone is 0-3 mm in extent (Pan et al. 2009). The antibacterial activity of the extract from *Tetrastigma* sp. was only weak, except at the highest concentration (200 µg /well) at which it was classified as having medium level of inhibition of *E. coli* growth. Extracts all other species were classified as having a strong inhibitory effect on the growth of *E. coli* bacteria at the highest concentration and for five of the species even at the lowest concentration of extract.

The results suggest that leaf extracts from at least eight of these medicinal plants have antibacterial potential presumably because of the phytochemically active compounds they contain. It is suspected that the antibacterial activity of the leaf extracts is due to the presence of secondary metabolite components such as terpenoids, steroids, saponins, tannins, and flavonoids (Sulastrianah et al. 2014). The extent of the antibacterial effect may vary with the way the particular extraction method influences the stability and effectiveness of these active compounds.

Table 5. Detailed evaluation of the toxic effects on *Artemia* shrimp larvae of extracts from three of the medicinal plant species at different extract concentrations ranging from 0.1% down to 0.001%

Sample	Concentration			Mort. LC50	Assessment
	%	µg/mL	(%)		
<i>Tetracera</i> sp.	0.1	1000	50	1030.99	Not toxic
	0.05	500	20		
	0.025	250	7		
	0.01	100	0		
	0.001	10	0		
<i>Bridelia glauca</i> Blume.	0.1	1000	50	1042.50	Not toxic
	0.05	500	20		
	0.025	250	7		
	0.01	100	7		
	0.001	10	0		
<i>Hippobroma longiflora</i> (L.) G. Don	0.1	1000	60	423.48	Toxic
	0.05	500	57		
	0.025	250	33		
	0.01	100	23		
	0.001	10	10		

In conclusion, sampled medicinal plant species traditionally used by the Kutai communities of Desa Sebelu Modern in East Kalimantan have been identified as containing bio-active compounds that are potentially beneficial in treating various diseases afflicting humans. Samples from nine species were shown to have significant antioxidant activity. The presence of phytochemicals compounds such as flavonoids, tannins, saponins, steroids, triterpenoids, and alkaloids was detected in most extracts from the species. The results of the toxicity assay showed that samples from one of the species were toxic, while nine sample was not toxic. The result of the antibacterial testing showed that nine species have a strong inhibitory effect on the growth of *E. coli* bacterial colonies while extracts of one species had only weak antibacterial activity.

Table 6. The effect of plants extracts from the ten medicinal plants on the growth of *E. coli* bacteria; the extent (mm) of the zone of inhibition of the bacterial colonies around wells in containing four concentrations of each extract.

Scientific name	Conc. (µg/well)	Inhibition zone (mm)	Anti-bacterial activity
<i>Callicarpa longifolia</i> Lam.	200	9.44	Strong
	100	9.00	Strong
	50	9.00	Strong
	25	8.22	Strong
<i>Tetracera</i> sp.	200	9.45	Strong
	100	0.00	Weak
	50	0.00	Weak
	25	0.00	Weak
<i>Bridelia glauca</i> Blume.	200	9.33	Strong
	100	8.67	Strong
	50	0.00	Weak
	25	0.00	Weak
<i>Tetrastigma</i> sp.	200	3.22	Medium
	100	3.00	Weak
	50	0.00	Weak
	25	0.00	Weak
<i>Leea indica</i> (Burm.f.) Merr.	200	12.00	Strong
	100	10.56	Strong
	50	10.00	Strong
	25	0.00	Weak
<i>Urena lobata</i> L.	200	11.89	Strong
	100	7.11	Strong
	50	6.67	Strong
	25	0.00	weak
<i>Clinacanthus nutans</i> (Burm.f) Lindau	200	12.67	Strong
	100	11.89	Strong
	50	11.78	Strong
	25	10.45	Strong
<i>Allophylus cobbe</i> (L.) Raeusch.	200	16.33	Strong
	100	14.67	Strong
	50	13.67	Strong
	25	10.44	Strong
<i>Alstonia iwahigensis</i> Elmer	200	12.44	Strong
	100	12.44	Strong
	50	12.44	Strong
	25	11.78	Strong
<i>Hippobroma longiflora</i> (L.) G. Don	200	20.67	Strong
	100	19.44	Strong
	50	17.89	Strong
	25	15.78	Strong

ACKNOWLEDGEMENTS

This paper reports a small part of the results of research funded by the Program for Doctoral Dissertation Grants, of the Directorate of Research and Community (DP2M), Directorate General of Higher Education; Ministry of Research, Technology and Higher Education, contract number – 058/SP2H/LT/ DPRM/IV/2017, 25 April 2017. The authors wish to convey sincere thanks for the funding support given. We also thank colleagues and all those who supported this research.

REFERENCES

- Achmad H. 1990. Traditional treatment of rural communities in East Kalimantan. Project Inventory and Cultural Values Development. Project Final Report. Directorate of History and Traditional Values, Directorate General of Culture, Ministry of Education and Culture, Jakarta [Indonesian]
- Arung ET, Muladi S, Sukaton E, Shimizu K, Kondo R. 2008. Artocarpin, a promising compound as whitening agent and anti-skin cancer. *J Trop Wood Sci Technol* 6: 33-36.
- Arunkumar S, Muthuselvam M. 2009. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World J Agric Sci* 5 (5): 572-576.
- Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Trop J Pharmaceut Res* 7 (3): 1019-1024.
- Berg H C. 2004. *Escherichia coli* in Motion. Springer, New York.
- Bhunia A. 2008. *Foodborne Microbial Pathogens*. Springer, New York.
- Boyd CE. 2005. LC_{50} calculations help predict toxicity. *Global Aquacult Advoc* Feb-2005: 84-87
- Cappuccino, J.G. and Sherman, N. 2001. *Microbiology: A Laboratory Manual*. 2nd Edition. The Benjamin Cummings Publishing Company. Rockland Community College, State University of New York, New York.
- Cefarelli G, Ambrosca BD, Fiorentino A, Izzo A, Mastellone C, Pacifica S, Piscopo V. 2006. Free-radical scavenging and antioxidant activities of secondary metabolites from reddened cv. Annurca apple fruits. *J Agric Food Chem* 54 (3): 803-809.
- Dvorak P, Benova K, Žďárský M, Sklenar Z, Havelkova A. 2010. Use of the crustacean *Artemia franciscana* for alternative biotests. *Acta Vet Brno* 79 (Suppl. 9): 47-53.
- Ebrahimzadeh MA, Nabavi SF, Nabavi SM. 2009. Essential oil composition and antioxidant activity of *Pterocarya fraxinifolia*. *Pak J Biol Sci* 12: 957-963.
- Evans WC. 2009. *Trease and Evans' Pharmacognosy*. 16th ed., Saunders Ltd., Edinburgh, UK.
- Hanani E, Mun'im A, Sekarini R. 2005. Identifikasi senyawa antioksidan dalam spons *Callispongia* sp. dari Kepulauan Seribu. *Majalah Ilmu Kefarmasian* 2 (3): 127-133. [Indonesian]
- Harborne JB. 1984. *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis*. Springer, Netherlands.
- Igwenyi IO, Offor CE, Ajah DA, Nwankwo OC, Ukaomah JI, Aja PM. 2011. Chemical composition of *Ipomoea aquatica* (green kangkong). *Intl J Pharm Biol Sci* 2 (4): 593-598.
- Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT. 2003. Comparison of antioxidant activities of isoflavones from kudzu root (*Pueraria lobata* Ohwi). *J Food Sci* 68 (6): 2117-2122.
- Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* 47 (10): 3954-3962.
- Khodadadi S, Nejadstari T, Naqinezhad A, Ebrahimzadeh MA. 2015. Diversity in antioxidant properties and mineral contents of *Allium paradoxum* in the Hyrcanian forests, Northern Iran. *Biodiversitas* 16 (2): 281-287.
- Kokate CK. 2001. *Pharmacognosy*. 16th ed. Nirali Prakasham, Mumbai, India.
- Mammadov R, Ili P, Ertem-Vaizogullar H. 2011. Antioxidant activity and total phenolic content of *Gagea fibrosa* and *Romulea ramiflora*. *Iran J Chem Chem Eng* 30 (3): 57-62.
- Manning SD. 2010. *Escherichia Coli Infections*. Infobase Publ., New York.
- McLaughlin JL, Chang CJ, Smith DL. 1993. Simple bench-top bioassays (brine shrimp and potato disk) for the discovery of plant antitumor compounds - Review of recent progress. In: Kinghorn AD, Baladrin MF. (eds). *Human Medicinal Agents from Plants*, Symposium Series No. 534. American Chemical Society, New York.
- Meyer BN, Ferrigni NR, Putman JE, Jacobson LB, Nichols DE, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* 45 (5): 31-34.
- Okwu CU. 2006. Evaluation of the phytonutrients, mineral and vitamin contents of some varieties of yam. *Intl J Mol Med Adv Sci* 12 (2): 199-203.
- Pan X, Chen F, Wu T, Tang H, Zhao Z. 2009. The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. *Food Control* 20 (6): 598-602.
- Pisoschi MA, Negulescu GP. 2011. Methods for total antioxidant activity determination: a review. *Biochem Anal Biochem* 1:1. 106. DOI: 10.4172/2161-1009.1000106.
- Potterat O. 1997. Antioxidants and free-radical scavengers of natural origin. *Current Org Chem* 1: 415-440.
- Saeed N, Khan MR, Shabbir M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extract *Torilis leptophylla* L. *BMC Compl Altern Med* 12: 221.
- Sampurno. 2003. Policy of Indonesian natural medicine development. 13th National Seminar on Indonesian Medicinal Plants. Pancasila University, Jakarta. [Indonesian]
- Sulastriana, Imran, Fitria ES. 2014. Inhibitory test of leaf extracts (*Annona muricata* L.) and sirih (*Piper betle* L.) against the growth of *Escherichia coli* bacteria. *Medula* 1 (2). [Indonesian]
- Wagner SL. 1993. Quarterly Report of EPA Grant #CR-821022-01, July 1, 1993 September. 30, 1993. National Pesticide Medical Monitoring Program. Oregon State University, Corvallis.