Potential of antioxidant and toxicity of some medical plants used by sub-ethnic communities of Bahau in East Kalimantan

P Rohim, E T Arung and I W Kusuma*

Faculty of Forestry, Mulawarman University, Samarinda, East Kalimantan, Indonesia. 75119.

*Corresponding author : kusuma_iw@yahoo.com

Abstract. The purpose of this research is to assay the potential antioxidant and toxicity of several plants from Bahau, a sub-ethnic in East Kalimantan in regard to their utilization as traditional medicines. This research includes phytochemical analysis, DPPH radical and superoxide radical scavenging activity as well as toxicity assay using Artemiasalina shrimp larvae. The results of the extraction showed the highest yield was 2.91% obtained from avung tanaq (Ficus uncinata), while the lowest is 1.14% obtained from tevoqsalah (Saccharum sp.) species. The result of phytochemicals showed that all plants contain alkaloid and carbohydrate. While carotenoids, saponins, triterpenoids and steroids were absence in all plant extracts. The DPPH radical scavenging activity test showed that the lowest IC$_{50}$ value of kayog kue (Dictamnus albus) by 23.96 μg/mL. The superoxide radical scavenging activity assay showed IC$_{50}$ values of all extract samples were >100 μg/mL. The toxicity assay showed that LC$_{50}$ values of all samples of extract tested were >1000 μg/mL. The present research suggested good potential activity of some plants from Bahau ethnic and further research oriented to wide uses of the plants as herbal products is needed.

1. Introduction

Antioxidant compounds are widely found in plants, both in flowers, leaves and fruits. Plants containing bioactive compounds such as flavonoids, alkaloids, and terpenoids are potential raw materials that can be used as natural antioxidants [1]. Exploration of medicinal plants as natural antioxidants is needed, especially in medicinal plants commonly used by rural communities. Indonesia has enormous potential in supplying raw materials of medicinal plants comprising 30,000 plant species from a total of 40,000 species of plants in the world, of which 940 are medicinal plants, accounting for 90% of the total number of medicinal plants in Asia [2]. Bahau is a local tribe in East Kalimantan, Indonesia. As a Dayak sub-tribe, the bahau community still uses medicinal plants as an alternative health care for some people. Types of diseases such as scabies, wounds, sore eyes, bone breakouts, arthritis, pregnant and postpartum care, diabetes, fever and others [3]. The purpose of this research is to assume the potential antioxidant and toxicity of several plants from Bahau, a sub-ethnic in East Kalimantan in terms of their utilization as traditional medicines.
2. Materials and Methods

2.1. Plant Materials
The bark and roots of medicinal plants were collected from the village of Matalibaq, East Kalimantan, Indonesia. The collected plants were: Dictamnus albus, Ficus uncinata, Saccharum sp, and Saprosmala tifolia. Plants were identified by, Raharjo Ari Suwasono, from the Laboratory of Forest Dendrology and Ecology, Mulawarman University and confirmed by reference. The voucher specimen was deposited at the Forest Products Chemistry Laboratory, at same university.

2.2. Preparation of Extracts
Plant samples ca 100 g were extracted with 96% ethanol at room temperature by continuously shaking with a shaker for 48 hours. Afterwards the extract solution was filtered by filter paper to get crude extract using a rotary evaporator at 40 °C. The gummy extracts then put in vacuum oven to near dryness to produce plant extracts.

2.3. Phytochemical analysis
Phytochemical analysis of the plant extracts was conducted by a standard procedure which included testing of alkaloid, flavonoid, carbohydrate, carotenoid, coumarin, tannin, saponin, triterpenoid and steroid [4,5,6].

2.4. Antioxidant assay

2.4.1. DPPH radical inhibition (1,1-diphenyl-2-picrylhydrazyl). Free radical scavenging activity against DPPH was done with reference to the method of [7]. Thirty three microliters of extract was diluted mixed by 467 μL and 500 μL DPPH. The final sample concentrations were 50, 25, 12.5, and 6.25 μg/mL. The solution was incubated for 20 min and absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as a positive control.

2.4.2. Superoxide radical inhibition. The antioxidant activity by means of radical scavenging activity was evaluated by superoxide radical scavenging assay with reference to [8]. 500 μL extract diluted was mixed by 500 μL NBT, 500 NADH μL and 50 μL PMS. The final sample concentrations were 50, 25, 12.5, 6.25, 3.125, and 1.56 μg/mL. The solution was incubated for 10 min and absorbance was measured at 560 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as a positive control.

2.5. Toxicity assay
Toxicity activity was evaluated by means of a brine shrimp lethality test using [9]. Plant extracts were tested at concentrations of 125, 250, 500, and 1000 μg/mL. After incubation 48 hours in room temperature (25-29 °C), nauplii (larvae) collected by pipette and used for testing. Survivors were counted after 24 hours.

The value of LC50 was obtained from the line corresponding to the line plotted concentration versus the lethal percentage.

3. Results and Discussion

3.1. Plant extracts
Four medicinal plants were extracted with ethanol at room temperature to give crude plant extract (Table 1).
Table 1. Yield of Medicinal Plant Extracts in Ethanol Solvents.

| No | Local Name     | Scientific Name | Part used | Yield % |
|----|----------------|-----------------|-----------|---------|
| 1  | AvungTanaq     | *F. uncinata*   | Root      | 2.91    |
| 2  | KayogKue       | *D. albus*      | Root      | 1.42    |
| 3  | KayuJeluh      | *S. latifolia*  | Stem Bark | 2.60    |
| 4  | Tevoq Salah    | *Saccharum sp.* | Root      | 1.14    |

3.2. Phytochemical analysis

The results on phytochemical screening of plant extracts were presented in Table 2. Phytochemical screening showed in all extracts containing alkaloids and carbohydrates, whereas flavonoids were only positive in the *F. uncinata* and *Saccharum* sp. Samples, the carotenoids were only positive in the *Saccharum* sp. latifolia. Tannin was only positive in the samples of *F. uncinata* and *S. latifolia*. In the analysis of saponin metabolite compounds, triterpenoids and steroids showed negative results in all extracts.

Table 2. Phytochemical analysis of Four Medicinal Plants

| No | Local Name     | Scientific Name | Part used | Phytochemicals |
|----|----------------|-----------------|-----------|-----------------|
|    |                |                 |           | Alk | Flav | Carbo | Car | Coum | Tan | Sap | Tri and Ste |
| 1  | AvungTanaq     | *F. uncinata*   | Root      | +   | +   | +     |       |       |     |     |       |
| 2  | KayogKue       | *D. albus*      | Root      | +   | -   | -     |       | +    | -   | -   | -     |
| 3  | KayuJeluh      | *S. latifolia*  | Stem Bark | +   | -   | +     |     |       | +   | - |       |
| 4  | Tevoq Salah    | *Saccharum sp.* | Root      | +   | +   | +     | +   | -    | -   | -   | -     |

Remarks: Alk= alkaloid; Flav= flavonoid; Carbo= Carbohydrate; Car= carotene; Coum= coumarin; Tan= tannin; Sap= saponin; Tri and Ste= triterpenoid or steroid.

The results of phytochemical analysis showed that the samples tested have the potential to be developed as traditional medicine, because they have secondary metabolite compounds that support for the cure of disease. Alkaloid is group of compound having some significant roles of biological activity such as ephedrine for asthma, analgesics for morphine, and anticancer effects of vinblastine [10]. Flavonoids are known to have anticancer agents, antiviral, antioxidant, antibacterial and anti-inflammatory [11]. Coumarin also showed to play important roles in many useful applications include, antioxidants [12], analgesic [13], anti-cancer [14], anti HIV [15], anti-inflammatory [16], antibiotics [17], anticoagulants [18] and anti tumor [19].
3.3. Antioxidant activity

3.3.1 Inhibition DPPH radical activity

Free radical inhibitory activity was evaluated by the DPPH radical scavenging mechanism. All samples tested showed an increase in inhibition by increasing concentration as presented in Figure 1. The best DPPH radical inhibition activity was demonstrated by a root sample of *D. albus* that caused inhibition of 82% at concentration of 50 µg/mL and was comparable to ascorbic acid with 95% inhibition. The IC\textsubscript{50} value presented in Table 3 shows the sample of *D. albus* with the lowest IC\textsubscript{50} value of 23.96 µg/mL. The result showed that *D. albus* possesses a very strong antioxidant as confirmed by [20].

**Figure 1.** Inhibition of DPPH Radical Percentage Graph

| No | Local Name   | Scientific Name | Part used | IC\textsubscript{50} (µg/mL) |
|----|--------------|-----------------|-----------|-----------------------------|
| 1  | AvungTanaq   | *F.uncinata*    | Root      | 49.36                       |
| 2  | KayogKue     | *D.albus*       | Root      | 23.96                       |
| 3  | KayuJeluh    | *S.latifolia*   | Stem Bark | 142.69                      |
| 4  | Tevoq Salah  | *Saccharum sp.* | Root      | 99.52                       |
|    | Ascorbic acid| -               | -         | 3.12                        |

**Tabel 3.** Results of DPPH radical inhibition activity assay
3.3.2. Inhibition of Superoxide Radical Activity

The mechanism of the assay is based on the reduction of nitro blue tetrazolium (NBT) in the presence of NADH (……) and phenazine methosulfate (PMS) under aerobic conditions. All samples tested showed low inhibitory activity as presented in Figure 2. *Saccharum sp.* and *S. latifolia* inhibited superoxide radical by 36% and 37.81% at concentration 50 μg/mL. The result also showed that the IC$_{50}$ values of the extracts were > 100 μg/ml.

![Figure 2](image)

**Figure 2.** Radical Scavenging Activity of Plant Samples against Superoxide Radical

| No | Local Name      | Scientific Name | Part of Plant | IC$_{50}$ (μg/mL) |
|----|-----------------|-----------------|---------------|-------------------|
| 1  | Avung Tanaq     | *F. uncinata*   | Root          | >100              |
| 2  | Kayog Kue       | *D. albus*      | Root          | >100              |
| 3  | Kayu Jeluh      | *S. latifolia*  | Stem Bark     | >100              |
| 4  | Tevoq Salah     | *Saccharum sp.* | Root          | >100              |
|    | Ascorbic acid   | -               | -             | 26.24             |

3.4. Toxicity

Toxicity test by using brine shrimp lethality test as presented in Table 5 showed that all samples had very low toxicity effects with values of LC$_{50}$ > 1000 μg/mL. A low LC$_{50}$ value will exhibit a high toxicity effect, while higher LC$_{50}$ indicates the low toxicity. According to [21] and [22], extracts having LC$_{50}$ values greater than 1000 μg/mL did not show significant toxicity to shrimp brines. This indicates that all of these extracts may not be toxic to humans.

| No | Local Name      | Scientific Name | Part used | Toxicity LC$_{50}$ (μg/mL) |
|----|-----------------|-----------------|-----------|-----------------------------|
| 1  | Avung Tanaq     | *F. uncinata*   | Root      | >1000                       |
| 2  | Kayog Kue       | *D. albus*      | Root      | >1000                       |
| 3  | Kayu Jeluh      | *S. latifolia*  | Stem Bark | >1000                       |
| 4  | Tevoq Salah     | *Saccharum sp.* | Root      | >1000                       |
4. Conclusion
The present result indicated that four medicinal plants include the Ficus uncinata, Dictamnus albus, Saprosmalatifolia and Saccharumsp. collected from the Bahau tribe in Matalibaqhave antioxidant potential. The result might be a scientific basis for the development of medicinal plants as natural antioxidant agents.

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References
[1] Purwanto D Bahri S Ridhay A 2017 Antioxidant Activity Test of Purnajiwa (Kapsia arborea Blume) Fruit Extract With Various Solvents Tadulako University KOVALENS (1) : 24-32
[2] APFORGEN 2010 Medicinal Plants Indonesia (in Indonesia) Asia Pasific Forest Genetic Resources Programme News Letter Edition 2 pp 1-4
[3] Yusro F Mariani Y Diba F 2014 Inventory of Medicinal Plants for Fever used by Four Dayak Sub-Ethnic in West Kalimantan Indonesia Kuroshio sci 8 pp 33-8
[4] Kokate C K Purohit A P Dan Gokhale S B 2001 Carbohydrate and Derived Products Drugs Containing Glycosides Drugs Containine Tannins Lipids and Protein Alkaloids Text Book of Pharmacognosy edition 7 pp 133-166 167-254 255-269 272-310 428-523
[5] Harborne JB 1998 Phytochemical Methods A Guide to Modern Techniques of Plant Analysis 3rd (India: Springer) pp 5-32
[6] Senthilmurugan G B Vasanthe K Suresh 2013 Screening and Antibacterial Activity Analysis of Some Important Medicinal Plants (India: Innovative Space of Scientific Research Journals) 2(2) pp 14652
[7] Shimizu K Kondo R Sakai K Takeda N Oniki T Novel 2001 Vitamin E derivative with 4-substituted resorcinol moiety has both antioxidant and tyrosinase inhibitory properties :Lipids (Japan: Springer) 36 (12) pp 1321–6
[8] Liu F VEC Ooi Chang S T 1997 Free radical scavenging activity of mushroom polysaccharide extracts Life Sci 60 pp 763–71
[9] Meyer H N 1982 Brine Shrimp Lethality Test (Amsterdam: Plant Research) Med 45 Hipokrates verlag Gmbrl pp 31-34
[10] Lu J J Bao J L Chen X P Huang M and Wang Y T 2012 Review Article Alkaloids Isolated From Natural Herbs as the Anticancer Agents Evidence-Based Complementary and Alternative Medicine Article ID 485042 12 Pages
[11] ZhenhuaY ZhangW Feng F Zhang Y and Kang W 2014a Glucosidase inhibitors isolated from medicinal plants(Beijing: Sciedencedirect) 3(3-4) pp 136–74
[12] Vazquez-Rodriguez S Figueroa-Guinez R Matos M J Santana L Uriarte E Lapier M Maya J D Olea-Azar C 2013 Synthesis of Coumarin-chalcone Hybrids and Evaluation of Their Antioxidant and Trypanocidal Properties Med Chem Commun 4 p 993
[13] Khode S Maddi V Aragade P Palkar M Ronad P K Mamledesai S Thippeswarany A Satyanarayana D 2009 Synthesis and Pharmacological Evaluation of a Novel Series of 5-(Substituted)aryl-3-(3-Coumarylnyl)-1-Phenyl-2-Pyrazolines as Novel Anti-inflammatory and Analgesic Agents Eur J Med Chem 44 p 1682
[14] Wu X Q Huang C Jia Y M Song B A Li J Liu X H 2014 Novel Coumarin–dihydropyrazole Thio-ethanone Derivatives : Design Synthesis and Anticancer Activity Eur J Med Chem 74 p 717
[15] Bhavsar D Trivedi J Parekh S Savant M Thakrar SBavishi A Radadiya A Vala H Lunagariya J Parmar M 2011 Synthesis and in Vitro Anti-HIV Activity of N-1,3-benzo[d]thiazol-2-yl-2-
(2-oxo-2H-chromen-4-yl) Acetamide Derivatives using MTT Method Bioorg Med Chem Lett 21 p 3443

[16] Timonen J M Nieminen R M Sareila O Goulas A Moilanen L J Haukka M Vainiotalo P Moilanen E Aulaskari P H 2011 Synthesis and Anti-inflammatory Effect of a Series of Novel 7-hydroxycoumarin Derivatives Eur J Med Chem 46 p 3845

[17] Chimenti F Bizzarri B Bolasco A Secci D Chimenti P Carradori S Granese A Rivanera D Lilli D Scaltrito M M 2006 Synthesis and in Vitro Selective anti-Helicobacter Pylori Activity of N-substituted-2-oxo-2H-1-benzopyran-3-carboxamides Fur J Med Chem 41 p 208

[18] Van S R M Wandelius M Kamali F Daly A K Manolopoulos V G De Boer A Barallon R Verhoef T I Kirchheiner J Haschke-Becher E 2009 Genotype-guided dosing of Coumarin Derivatives: The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) Trial Design Pharmacogenomics 10 p 1687

[19] Gouda M A Berghot M A Baz E A Hamama W S 2012 Synthesis Antitumor and Antioxidant Evaluation of Some New Thiazole and Thiophene Derivatives Incorporated Coumarin Motety Med Chem Res 21 p 1062

[20] Molyneux P 2004 The use of The Stable Free Radical Diphenylpicryl hydrazyl (DPPH) For Estimating Antioxidant Activity Songklangkarin J Sci Technol 26 (2) pp 211-9

[21] Mbwanbo Z H Moshi M J Masimba P J Kapingu M C Nondo RS O 2007 Antimicrobial activity and brine shrimp toxicity of extracts of Terminalia brownii roots and stem BMC Compl Altern Med 7:9 doi 10.1186/1472-6882-7-9

[22] Moshi M J Innocent E Magadula J J Otieno D F Weisheit A Mbabazi P K Weisheit A Nondo R S O 2010 Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region north western Tanzania Tanzania J Health Res 12 (1) pp 63-7