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Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia

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Abstract

Some selected medicinal plants used by Bentian tribe from Indonesia were evaluated for potential antimicrobial and antioxidant properties. The leaves stem bark and root of the plants were extracted with *n*-hexane, ethyl acetate and ethanol to give respective extracts. Antimicrobial activity against *Propionibacterium acnes* and *Candida albicans* was determined by an agar well diffusion method. Antioxidant activity was assayed by the DPPH radical scavenging activity mechanism. The results showed that *n*-hexane, ethyl acetate and ethanol extracts of *Cananga odorata*, ethanolic extract of *Chromolaena odorata*, and ethanolic extracts of *Hyptis capitata* and *Ampelocissus cinnamomeae* displayed good activity against *P. acnes* at 25–400 μg/well of the extracts tested. The *n*-hexane and ethanol extracts of *Chromolaena odorata* and *H. capitata*, and the *n*-hexane, ethyl acetate and ethanol extracts of *Cananga odorata* displayed more activity against *C. albicans* than others at 25–400 μg/well of the extracts tested. The most antioxidant activities against DPPH were displayed by the ethanol extracts of *Ficus variegata* stem bark, *Leucosyke quadrinerva* root and *Clausena excavata* leaves exhibiting 91%, 91% and 86% inhibition, respectively. The present results showed potential of some medicinal plants used by Bentian tribe from Indonesia as natural anti-microbial and anti-oxidant and potentially anti-inflammatory agents.
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Keywords: Antimicrobial activity; Antioxidant activity; Natural products; Phytochemicals; Medicinal plants

1. Introduction

Natural products are defined as natural sources-derived substances having biological activities. Natural products have long been implemented as alternative health care treatment and in discovery of modern drugs [1]. A major focus of natural product chemistry has been toward drug design and discovery. Our literature tracking displayed that most research activity into natural products in Indonesia is still limited to the inventory of folkloric information and utilization of various plants and trees, meaning that obtaining scientific proof for their biological activity is still challenging [2,3].

Medicinal plants refer to the class of plants applied for therapy or to possess pharmacological actions for human and animal. In morphological aspect, there is no difference between medicinal and others plants, except the characteristics of certain plant to exhibit medicinal benefits. Indonesia comprises about 110 million hectares and serves about 80% world medicinal plants. It is estimated that 28,000 plant species exist in Indonesian forest. Of these, 7,000 species are medicinal plants, which is equal to 90% of medicinal plants in Asia. So far, 1000 species have been known and utilized in traditional medicine [4].

In terms of the Indonesian medicinal plant diversity, more than 250 medicinal plant species from 165 genera and 75 family have been used by Dayak Ransa tribe in West Kalimantan, Indonesia. About 200 species of forest medicinal plants have been used by Kenyah Dayak community in Apo Kayan plateau in Indonesia. Furthermore, it has been reported that there are 62 medicinal plants species used by Dayak Benuaq community in West Kutai, Indonesia [5–7].

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In the last decade, the demand for antimicrobial agents is increasing due to emergent clinical microbial strains resistant to one or several antibiotics [8]. Plants promise a source of natural antimicrobial agents. It has been reported that the antimicrobial activity of plants is related with the defense mechanism against microorganism [9]. Other applications for natural antioxidants may include bioactive nutraceuticals, bio-pharmaceuticals, and food additives. In relation to that, the extraction, characterization and utilization of natural antioxidants are intensively performed to find potent candidates in combating the aging process [10,11].

Bentian is a local tribe exists in East Kalimantan, Indonesia. As a sub tribe of the Dayak, the Bentian's community still uses medicinal plants as an alternative healthcare treatment for several types of disease such as scabies, wounds, sore eyes, broken bones, arthritis, treatment of pregnant and postpartum mothers, diabetes, fever and others [12]. Despite the extensive uses, there have been only limited attempts to explore the biological properties of the plants in relation to their medicinal uses. Here, we present data on antimicrobial and antioxidative activities of ten medicinal plants collected from the Bentian tribe in Indonesia.

2. Materials and methods

2.1. Plant materials and chemicals

Leaves, stem bark and/or root of medicinal plants were collected from Tende village, East Kalimantan, Indonesia, in May 2014. The selected plants were: *Ampelocissus cinnamomea*, *Clausena excavata*, *Fordia splendidissima*, *Hyptis capitata*, *Hemigraphis alternate*, *Chromolaena odorata*, *Pogostemon* sp., *Amischotolype monosperma*, *Leucosyke quadrinervia*, *Ficus variegata*, and *Cananga odorata*. The plants were identified by a taxonomist, Dr. Medi Hendra of Mulawarman University and confirmed by references. Voucher specimens were deposited in the Laboratory of Forest Products Chemistry, Faculty of Forestry, Mulawarman University. The plant materials were shade dried for 3 days and ground with a blender. DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). DMSO (dimethyl sulfoxide), sulfuric acid, hydrochloric acid, acetic anhydride, potassium iodide and peptone were purchased from Merck (Darmstadt, Germany). Ascorbic acid, 1-naphtol and bismuth (III) nitrate were obtained from Sigma (St. Louis, MO, USA). Nutrient broth was obtained from Difco (Detroit, MI, USA). Other chemicals were of HPLC grade or the highest purity commercially available.

2.2. Extraction

Ground plant samples (4–19 g) were extracted successively with *n*-hexane, ethyl acetate and ethanol at room temperature with continuous shaking on a shaker (7400 Tübingen; Edmun Buchler, Germany) for 48 h. This process was then repeated. Following filtration of the suspension through Whatman filter paper No. 2 (Maidstone, UK), the crude alcohol extracts were rotoevaporated at 40 °C and put in a vacuum oven to near dryness to yield the plant extract as listed in Table 1.

2.3. Antimicrobial assay

Antimicrobial assays were conducted using the agar well disk diffusion method as previously reported [13] with slight modification. *Propionibacterium acnes* and *Candida albicans* were used in all experiments. Nutrient agar was used as media. Twenty-milliliter aliquots of sterile media were transferred to Petri dishes and allowed to solidify. The media plates were inoculated with 20 μL of microbial suspension spread uniformly on the surface of the plates. A seven-mm well were cut using a sterile cork borer and 20 μL acetone solution containing 25–400 μg extracts were added to the well. Chloramphenicol was used as a positive control at the concentration of 10 μg/20 μL in each well. The plates were incubated in the dark at 32 °C for 24 h. Zones of inhibition around the well were measured in mm. Activity index (AI) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug [13].

2.4. Antioxidant assay

The sample was first dissolved in DMSO and used at a 30 times dilution for the actual experiment. The DPPH radical scavenging method was performed as previously described by Shimizu et al. [14]. UV absorption was measured on a Shimadzu UV-VIS 1240 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

3. Results and discussion

3.1. Plant extracts

Leaves, stem bark and/or root of ten medicinal plants from were macerated by *n*-hexane, ethyl acetate and ethanol at room temperature, successively (Table 1). The *n*-hexane maceration of the plants yielded 0.45–8.01% extracts on the basis of sample dry weight. *C. excavata* root gave the lowest yield, while *A. monosperma* leaves yielded the highest percentage of extract. The ethyl acetate maceration gave 0.55–10.63% extracts on the basis of sample dry weight. *L. quadrinervia* root yielded the lowest percentage of extract, while the highest one was obtained from *Chromolaena odorata* leaves. The ethanol maceration gave 1.88–20.19% extracts. *A. cinnamomea* leaves gave the lowest percentage of extract, while the highest percentage was obtained by *H. capitata* root.

3.2. Antimicrobial activity

The development of microbial resistance to presently available antibiotics led the search for new antimicrobial agents [15]. Due to the problem of microbial resistance to antibiotics, attention is given toward biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antimicrobial activities [16]. Our search for antimicrobial bioactivity from tropical medicinal plants revealed antimicrobial activity of ten medicinal plants used by Bentian tribe in Indonesia. Results of antimicrobial tests of the plant extracts are listed in Table 2.

Table 1
Yield of medicinal plant extracts in several solvents.

No.	Sample name	Plant part	*n*-Hexane		Ethyl acetate		Ethanol	
			Extract yield (g)	Percentage (%)	Extract yield (g)	Percentage (%)	Extract yield (g)	Percentage (%)
1	*A. cinnamomea*	Leaves	0.18	2.09	0.26	3.04	0.17	1.88
2	*C. excavata*	Root	0.03	0.45	0.08	1.33	0.15	2.53
3	*C. excavata*	Leaves	0.39	2.26	1.58	9.11	0.69	3.98
4	*F. splendidissima*	Root	0.36	1.96	0.52	2.84	0.53	2.89
5	*H. capitata*	Root	0.03	0.70	0.07	1.80	0.76	20.19
6	*H. alternate*	Leaves	0.10	1.15	0.12	1.39	0.69	7.98
7	*Chromolaena odorata*	Leaves	0.80	4.45	1.92	10.63	1.52	8.46
8	*Pogostemon* sp.	Leaves	0.16	2.07	0.42	5.83	1.50	20.00
9	*A. monosperma*	Leaves	0.69	8.01	0.09	1.10	0.26	3.13
10	*L. quadrinervia*	Root	0.41	4.31	0.05	0.55	0.65	6.70
11	*F. variegata*	Bark	0.58	3.36	0.40	2.29	0.83	4.75
12	*Cananga odorata*	Bark	0.29	2.18	0.57	4.23	0.39	2.88

Table 2
Antibacterial activity of the collected medicinal plants againts *Propionibacterium acnes*.

No.	Plant sample	Plant parts	Extracts	Inhibition zone (mm)/extract tested (μg/well)					
				(−)	25	50	100	200	400
1	*A. cinnamomea*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	10 ± 0.58	12 ± 0.33	12 ± 0.67	12 ± 1.17	15 ± 1.67
2	*C. excavata*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	8 ± 0.77	9 ± 1.20	9 ± 0.51	9 ± 0.38	10 ± 0.33
3	*C. excavata*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	1 ± 0.05	1 ± 0.03	1 ± 0.05	2 ± 1.45	2 ± 0
4	*F. splendidissima*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
5	*H. capitata*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	14 ± 0	16 ± 0.58	16 ± 0.51	16 ± 0.71	16 ± 1.02
6	*H. alternate*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	1 ± 0.45	1 ± 0.54	2 ± 0.19	2 ± 0.35	2 ± 0.35
7	*Chromolaena odorata*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	12 ± 0.51	13 ± 0.96	14 ± 0.58	15 ± 0.51	17 ± 1.67
8	*Pogostemon* sp.	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	1 ± 0.31	1 ± 0.35
9	*A. monosperma*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
10	*L. quadrinervia*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
11	*F. variegata*	Stem bark	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
12	*Cananga odorata*	Stem bark	*n*-Hexane	0	8 ± 0.38	10 ± 0.33	11 ± 0.19	12 ± 0.67	13 ± 0.33
			EtOAc	0	7 ± 0.33	7 ± 0.51	9 ± 0.51	11 ± 0.19	12 ± 0.69
			EtOH	0	13 ± 0.38	14 ± 0.19	16 ± 0.58	16 ± 0.51	19 ± 1.58
	CHP						30 ± 0.33		

Remarks: Inhibition zones are presented as mean of triplicates. Inhibition zones (IZ) include the well diameter (7 mm), (−), negative control (acetone only); *Abbreviations*: EtOAc, ethyl acetate; EtOH, ethanol; CHP, chloramphenicol (10 μg/well).

Table 3
Antimicrobial activity of the collected medicinal plants againts *Candida albicans*.

No.	Plant sample	Plant parts	Extracts	Inhibition zone (mm)/extract tested (μg/well)					
				(−)	25	50	100	200	400
1	*A. cinnamomea*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
2	*C. excavata*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
3	*C. excavata*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
4	*F. splendidissima*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
5	*H. capitata*	Root	*n*-Hexane	0	13 ± 0.19	14 ± 0.33	14 ± 0.51	16 ± 0.19	16 ± 0.58
			EtOAc	0	0	0	0	0	0
			EtOH	0	16 ± 0.84	17 ± 0.84	17 ± 0.51	19 ± 0.51	19 ± 0.33
6	*H. alternate*	Leaves	*n*-Hexane	0	8 ± 0.58	9 ± 1.17	9 ± 1.20	9 ± 0.33	9 ± 0.19
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
7	*Chromolaena odorata*	Leaves	*n*-Hexane	0	0	9 ± 0.33	11 ± 1.50	13 ± 1.58	15 ± 1.45
			EtOAc	0	0	0	0	0	0
			EtOH	0	11 ± 0.33	14 ± 0.38	16 ± 0.33	18 ± 0.77	19 ± 0.84
8	*Pogostemon* sp.	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
9	*A. monosperma*	Leaves	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
10	*L. quadrinervia*	Root	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
11	*F. variegata*	Stem bark	*n*-Hexane	0	0	0	0	0	0
			EtOAc	0	0	0	0	0	0
			EtOH	0	0	0	0	0	0
12	*Cananga odorata*	Stem bark	*n*-Hexane	0	12 ± 0.88	15 ± 1.50	17 ± 1.58	17 ± 0.67	17 ± 0.84
			EtOAc	0	14 ± 0	15 ± 0	16 ± 0	16 ± 0	17 ± 0.69
			EtOH	0	6 ± 0.51	9 ± 0.33	10 ± 0.84	13 ± 0.69	16 ± 1.02
	NYS						30 ± 0.15		

Remarks: Inhibition zones are presented as mean of triplicates. Inhibition zones (IZ) include the well diameter (7 mm), (−), negative control (acetone only); *Abbreviations*: EtOAc, ethyl acetate; EtOH, ethanol; NYS, nystatin 10 μg/well.

In the assay against *P. acnes*, 4 plant extracts, i.e. *n*-hexane, ethyl acetate and ethanol extracts of *Cananga odorata*, ethanolic extract of *Chromolaena odorata*, and ethanolic extracts of *H. capitata* and *Ampelocissus cinnamomeae* displayed good activity at 25–400 μg/well. The ethanolic extract of *Cananga odorata* showed the highest effect with activity indices (AI) of 0.43 and 0.63 against *P. acnes* relative to chloramphenicol, a standard drug (Table 2). *P. acnes*, a Gram-positive anaerobic bacterium, is part of the normal human skin flora and is involved in inflammatory diseases, such as acne vulgaris [17]. *P. acnes* has also been reported to play an important role in other human diseases, including infections of indwelling medical devices [18,19], and potential roles in the etiology of sarcoidosis [20] and prostate cancer [21]. The results suggested the possibility that the extracts maybe used for treating acne lesion, indwelling medical devices infection, prostate cancer and other acne-related diseases.

In the anti-candidal assay (Table 3), *n*-hexane and ethanol extracts of *Chromolaena odorata*, *H. capitata* and *Cananga odorata* showed potent activity to inhibit *C. albicans* growth. The ethanol extract of *Chromolaena odorata* displayed the highest activity against *C. albicans* with AI of 0.37–0.63 relative to nystatin, a commercial antibiotic for treating Candida infection. *C. albicans* is thought to be the major fungal pathogen of humans [22]. *C. albicans* accounts for approximately 50% of cases of candidemia associated with colonization of indwelling devices, such as catheters, endotracheal tubes, and pacemakers [23,24]. Furthermore, infections caused by *C. albicans* remain the predominant nosocomial fungal infections, due to the increasing population of patients whose immune systems are compromised by AIDS or immunosuppressant or anticancer therapy [25,26]. Limited number of available antifungal drugs and repeated exposure to these limited antifungal agents led to the rapid development of drug resistance [27]. Potent activity of the plant extracts against *C. albicans* suggests the possibility for the treatment of candidemia, nosocomial infection, and other Candida infection-caused diseases.

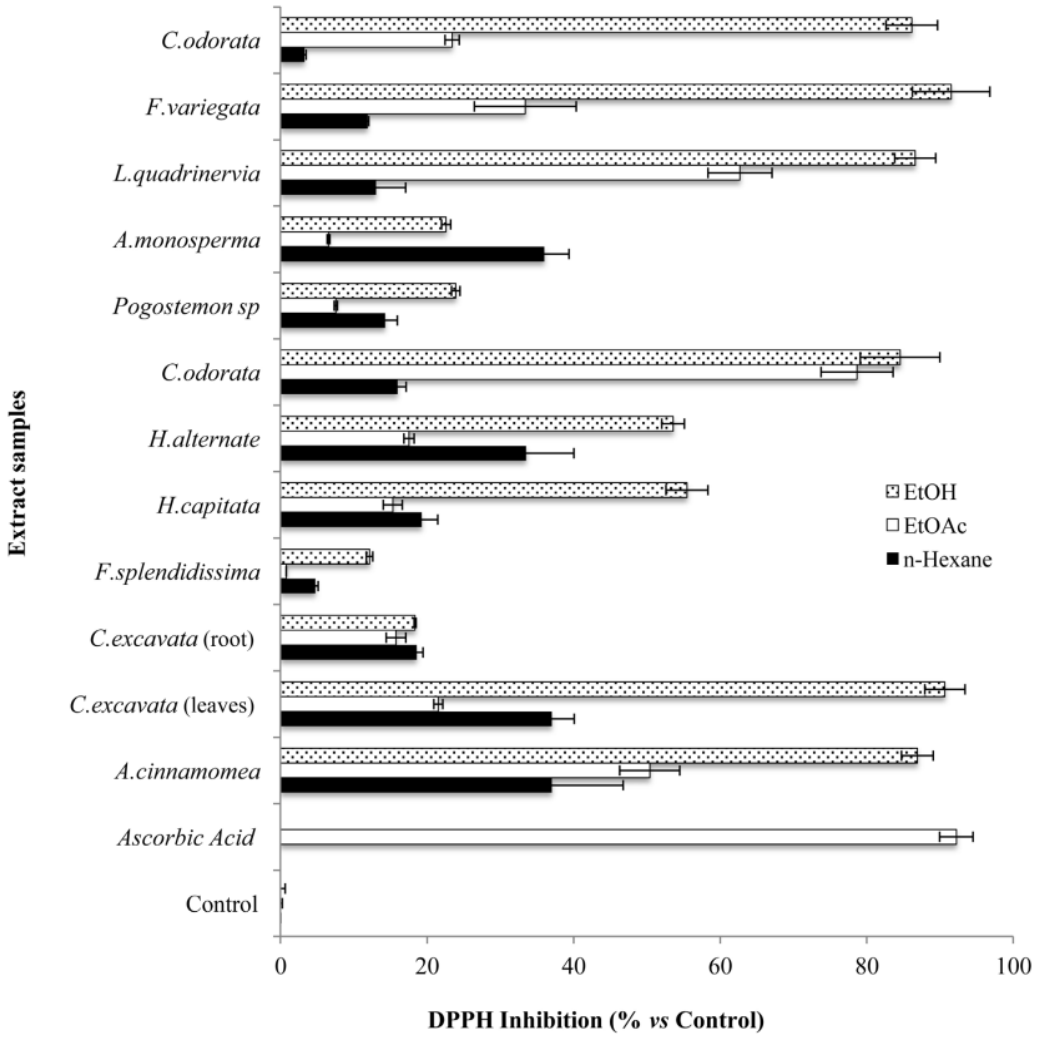

Fig. 1. Antioxidant activity of the collected medicinal plant extracts against DPPH at 50 ppm.

3.3. Antioxidant activity

The antioxidant activity of the plant extracts was evaluated by DPPH radical scavenging mechanism. DPPH is a free radical compound that has widely been used to test the free radical scavenging abilities of various types of samples [28,29]. The antioxidant activities of leaves, stem bark and/or root of the collected medicinal plants are given in Fig. 1. The results are shown as the relative activities against the standard ascorbic acid. The results showed that for *n*-hexane extracts tested at 50 ppm, the highest antioxidant activity was displayed by *C. excavata* leaves causing 37% DPPH inhibition. Ethyl acetate extracts of *C. odorata* stem bark showed strongest antioxidative effects causing 79% DPPH inhibition. While the highest antioxidant activity of ethanolic extracts was displayed by *F. variegata* stem bark and *Leucosyke quadrinerva* root causing 91% DPPH inhibition. Overall, the most antioxidant activities against DPPH were displayed by the ethanol extracts of *F. variegata* stem bark, *L. quadrinerva* root and *C. excavata* leaves showing 91%, 91% and 86% inhibition at the concentration of 50 ppm. In comparison, the standard ascorbic acid showed 92% DPPH inhibition in the assay.

The screening and characterization of antioxidants derived from natural sources has gained much attention and efforts have been put into identifying compounds as suitable antioxidants to replace synthetic ones [30]. The search for phytochemicals with potent antioxidant continues to be of great importance in the search for remedies against free radical-mediated diseases, prevention of oxidative reactions in foods, protection against DNA damage and carcinogenesis, and possible substances with wide range of pharmacological activities such as anti-inflammatory, anti-bacterial, and anti-fungal properties

[31,32]. Further investigation on isolation and characterization of bioactive compounds derived from natural extracts is in progress.

4. Conclusions

The present work has proved that the extracts of leaves, stem bark, and/or root of several plants used by Bentian tribe in Indonesia possessed strong antimicrobial and antioxidant properties. The results serve as a scientific basis to further develop some of those Indonesian species into medicinal plants.

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