RESEARCH ARTICLE | JULY 21 2016

Anticandidal activity of several plants used by Bentian tribe in East Kalimantan, Indonesia FREE

Irawan Wijaya Kusuma; Nur Maulida Sari; Murdiyanto; Harlinda Kuspradini

AIP Conf. Proc. 1755, 040002 (2016)
https://doi.org/10.1063/1.4958477

Anticandidal Activity of Several Plants Used by Bentian Tribe in East Kalimantan, Indonesia

Irawan Wijaya Kusuma[1,a)], Nur Maulida Sari[1], Murdiyanto[1] and Harlinda Kuspradini[1]

[1]*Laboratory of Forest Products Chemistry, Faculty of Forestry, Mulawarman University, Kampus Gn. Kelua, Jl. Ki Hajar Dewantara, Indonesia 75116*

[a)] Corresponding author: kusuma_iw@yahoo.com

Abstract. Research on the potential of diversity, ethnobotany and ethnopharmacology and bioactivity of plants used by local tribes in Kalimantan is still relatively inadequate. This research aimed to evaluate the antimicrobial activity of medicinal plants used by the Bentian tribe in East Kalimantan. In this study, we used 19 medicinal plants collected from our previous study. From these samples, we tested 21 extracts derived from *n*-hexane, ethyl acetate and ethanol as representative of different polarity solvents. The Anticandidal activity was examined using agar well diffusion against *Candida albicans*. Anticandidal activity of the extracts was evaluated by relative comparison with the standard antibiotic, chloramphenicol. The results showed that among the *n*-hexane extracts, those derived from *Blumea balsamifera* and *Triumfettar homboidea* exhibited the highest inhibition activity on the growth of *C. albicans* by 27-56%. The ethyl acetate extract of *B. balsamifera* and *Stachytarpheta jamaicensis* observed to have the highest anticandidal activity with the inhibition value of 39-57%. The ethanol extracts of *Vernonia arborea* and *B. balsamifera* showed potent activity to inhibit the *C. albicans* growth with 35-60% inhibition activity. Based on the results, it can be concluded that *B. balsamifera*, *V. arborea* and *S. jamaicensis* were the most active species to exhibit anticandidal activity and promising source to be developed as natural anticandidal agents.

INTRODUCTION

Drug design and discovery has been a major focus of natural product chemistry research. To the best of our knowledge, advance research into natural products of Indonesian medicinal plants is limited. The research activity in medicinal plants in Indonesia is limited to the inventory of folkloric information and utilization of various plants and trees. It means research into the scientific proof for their biological activity of the local medicinal plants is still challenging [1, 2].

Medicinal plants refer to the class of plants applied for therapy or to possess pharmacological actions for human and animal. In a morphological aspect, there is no difference between medicinal and others plants, except the characteristics of some typical plant possessing medicinal purposes. So far, 80% world medicinal plants have been reported to exist in Indonesia. About 28,000 plant species are existed in Indonesia and 7,000 species have been known as medicinal plants. So far, 1.000 species have been known and utilized in traditional medicine [3-4].

Regarding the plants and ethnic diversities in Indonesia, Dayak Ransa tribe in west Kalimantan, Indonesia use more than 250 medicinal plant species that belong to 165 genera and 75 families Dayak Kenyah in Apo Kayan plateau, Indonesia was reported to use about 200 plant species as traditional medicines. Furthermore, about 62 medicinal plants have been known and used by Dayak Benuaq in West Kutai, Indonesia [5-6].

Microbial resistance to one or several antibiotics have led to the increase of the demand for effective antimicrobial agents [7]. Plants promise a source of natural antimicrobial agents. It has been reported that the antimicrobial activity of plants is related to the defense mechanism against microorganism [8]. 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

Advances of Science and Technology for Society
AIP Conf. Proc. 1755, 040002-1–040002-6; doi: 10.1063/1.4958477
Published by AIP Publishing. 978-0-7354-1413-6/$30.00

bones, arthritis, treatment of pregnant and postpartum mothers, diabetes, fever and others [9]. Despite the extensive uses, there have been only limited attempts to explore thebiological properties of the plants in relation to their medicinal uses. Here, we reported anticandidal activity of 19 medicinal plant specied collected from the Bentian tribe in Indonesia.

MATERIALS AND METHODS

Plant materials and chemicals

Leaves, fruit, stem bark and/or root of medicinal plants were collected from Tende village, East Kalimantan, Indonesia, in May 2014. The selectedplants were: *Macaranga gigantea*, *Poikilospermum suaveolens, Ceiba pentandra, Fibraurea tinctoria, Ficus* sp*., Gonocaryum* sp*., Mallotus mollissimus, Triumfettar homboidea, Cocos nucifera, Vitex pinnata, Scoparia dulcis, Stachytarpheta jamaicensis, Cordyline fruticosa, Excoecariaco chinchinensis, Sidarhom bifolia, Vernonia arborea, Erythrina* sp*., Blumea balsamifera* and *Artocarpus altilis*. The collected plants were determined their identity by confirmation to a taxonomist in Mulawarman University. Voucher speciments were kept in our laboratory for further reference. The plant materials were prepared by drying for 3 days under the shade and milled with a blender. The nutrientbroth was obtained from DIFCO (Detroit, MI, USA). Other chemicals were of HPLC grade or the highest purity commercially available.

Extraction

Powdered plant samples (4-20 g) were extracted successively with *n*-hexane, ethyl acetate and ethanol at room temperature with continuous shaking on a shaker (7400 Tübingen; EdmunBuchler, Germany) for 48 hours. This process was repeated for three times. Subsequently, the suspensions have filtrated through Whatman filter paper No. 2 (Maidstone, UK). The respective obtained extracts were evaporated in a rotary evaporator at 40ºC and put in a vacuum oven to near dryness to yield the plant extract as listed in Table 1.

Antimicrobial assay

The anticandidal assay was conducted using the agar well disc diffusion method as previously reported [10] with slight modification. *Candida albicans* was used in all experiments with nutrient agar 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 hours. Zones of inhibition around the well were measured, and the anticandidal activity (AA) was calculated as the mean inhibition zone for test sample divided by the mean inhibition zone for the standard drug [10].

RESULTS AND DISCUSSION

The bioactivity study of leaves, fruit, stem bark and/or root of 19 medicinal plants collected from Bentian tribe at Tende village, East Kalimantan,was done for their antimicrobial activity against *C. albicans,* a human pathogenic yeast likefungi. The leaves, fruit, stem bark and/or root of the plant were macerated by *n*-hexane, ethyl acetate and ethanol at room temperature, successively (Table 1). The *n*-hexane maceration of the plants yielded 0.26 - 4.29% extracts of dry weight sample. Stembark of *C. pentandra* gave the lowest yield while the leaves yielded the highest percentage of extract. The ethyl acetate maceration gave 0.09 - 18.04% extracts of dry weight sample .*B. balsamifera* yielded the lowest yield of extract while the highest one was obtained from *M. gigantea* leaves. The ethanol maceration gave 0.21 - 26.73% extracts. *C. pentandra* stem bark gave the lowest percentage of extract while the highest yield was obtained from *V. pinnata* fruit.

TABLE 1. The Yield of Extracts of Medicinal Plants Traditionally Used by Bentian Tribe.

No	Samples name	Plant part	Yield (%)*		
			n-hexane	Ethyl acetate	Ethanol
1	*Macaranga gigantea*	Leaves	1.66	18.04	11.28
2	*Poikilospermum suaveolens*	Fruit	0.50	3.35	8.86
3	*Ceiba pentandra*	Leaves	4.29	7.36	5.01
4	*Ceiba pentandra*	Stem bark	0.26	0.32	0.21
5	*Fibraurea tinctoria*	Root	0.42	1.22	1.60
6	*Ficus* sp.	Leaves	1.88	3.48	3.50
7	*Gonocaryum* sp.	Leaves	0.35	2.53	2.38
8	*Mallotus mollissimus*	Root	0.65	0.55	3.18
9	*Triumfettar homboidea*	Root	0.88	1.30	4.22
10	*Cocos nucifera*	Root	0.45	0.36	6.17
11	*Vitex pinnata*	Fruit	0.31	4.89	26.73
12	*Scoparia dulcis*	Leaves	0.85	1.66	11.32
13	*Stachytarpheta jamaicensis*	Leaves	0.81	2.05	9.54
14	*Cordyline fruticosa*	Root	0.42	0.29	7.53
15	*Cordyline fruticosa*	Leaves	1.48	10.50	10.87
16	*Excoecariaco chinchinensis*	Stem bark	2.19	2.50	3.25
17	*Sidarhom bifolia*	Leaves	0.63	1.48	2.56
18	*Vernonia arborea*	Leaves	2.47	7.06	6.19
19	*Erythrina* sp.	Stem bark	0.40	0.65	2.23
20	*Blumea balsamifera*	Leaves	2.76	0.09	3.91
21	*Artocarpus altilis*	Leaves	0.46	15.38	3.98

*Yield was calculated on the basis of dry weight of the plant samples

Antimicrobial activity was conducted on 21 extracts obtained from leaves, fruit, stem bark or root parts of 19 plants. Table 2 shows the inhibitory effect of *n*-hexane extract on the *C. albicans* growth. Among the extracts tested, 13 extracts (62%) were capable to inhibit the growth of fungal *C. albicans* causing 6-10 mm of inhibition zone at 25 µg/well of extract applied to the well. The results showed a potenty of the plant extracts as natural antibiotic to control the growth of *C. albicans*. At 400 µg/well of applied extract, 19 of 21 extracts (90%) showed potential inhibition the growth of *C. albicans*. *M. gigantea* and *C. nucifera*extract shown to be the weakest while the strongest activity was shown by *B. balsamifera* extract at the low amount of extract tested. Antimicrobial activity (AA) of the extracts tested at 25 µg/well is ranged from 24-36% relative to chloramphenicol as a standard antibiotic. At 400 µg/well, the plant extracts had 31-59% of antimicrobial activity relative to the standard. At the highest amount of extract tested, *T. rhomboidea* caused 16 mm inhibition zone, that was equal to 59% of antimicrobial activity relative to the standard .The results showed that antimicrobial activity of the collected plant extract was on the concentration-dependent basis. It means increasing the amount of extracts applied to the well caused the better inhibition zones and antimicrobial activity.

The inhibitory effect of ethyl acetate extracts on the *C. Albicans* growth was presented in Table 3. Among 21 extracts tested, 12 extracts (57%) inhibited the growth of *C. albicans* at 25 µg/well of the extracts applied to the media. As displayed by *n*-hexane extract, the high percentage of active extract also promised the potential uses of the plant as a natural anticandidal agent. Furthermore, at the highest amount of applied extracts, 20 plant extracts (95%) displayed potential inhibitory activity. As displayed by *n*-hexane extract, *B. balsamifera* exhibited the highest inhibition by causing 9 mm inhibition at 25 µg/well of extract applied to the well. *Gonocaryum*s p was shown to be the lowest by causing 6 mm of inhibition zone. At the highest amount of extract applied to the well, *S. jamaicensis* caused 14 mm of inhibition zone that is equal to 57% of antimicrobial activity relative to chloramphenicol.

The results of anticandidal activity assay of the ethanol extracts were displayed in Table 4. At the minimum amount of applied extract (25 µg/well), 13 extracts (62%) showed to be active. Furthermore, at the maximum amount of applied extracts, among 21 plant extracts, 19 extracts (90%) displayed potential inhibitory activity. At 25 µg/well, *V. arborea* exhibited the highest inhibition by causing 10 mm inhibition. *S. rhombifolia* was shown to be the less active by causing 5 mm of inhibition zone. At the highest amount of extract applied to the well, *B. balsamifera* caused 17 mm of inhibition zone that is equal to 60% of antimicrobial activity relative to chloramphenicol.

TABLE 2. Anticandidal Activity of *n*-Hexane Extracts of Medicinal Plants from Bentian Tribe.

No	Samples	Amount of extracts applied									
		25 µg/well		50 µg/well		100 µg/well		200 µg/well		400 µg/well	
		IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)
-	Acetone (control)					0					
-	CHP (20 µg/well)					100					
1	*Macaranga gigantea*	0	0	7	26	8	29	9	34	11	40
2	*Poikilospermum suaveolens*	0	0	0	0	0	0	0	0	0	0
3	*Ceiba pentandra*	0	0	0	0	0	0	0	0	0	0
4	*Ceiba pentandra*	0	0	0	0	8	32	10	39	13	48
5	*Fibraurea tinctoria*	0	0	0	0	0	0	6	24	8	31
6	*Ficus* sp.	0	0	0	0	0	0	9	33	11	42
7	*Gonocaryum* sp.	0	0	0	0	0	0	10	38	12	45
8	*Mallotus mollissimus*	0	0	0	0	0	0	9	33	11	40
9	*Triumfettar homboidea*	8	30	10	38	11	41	13	48	16	59
10	*Cocos nucifera*	6	24	7	28	8	30	9	35	11	43
11	*Vitex pinnata*	0	0	0	0	0	0	0	0	0	0
12	*Scoparia dulcis*	7	28	9	34	10	38	2	44	13	51
13	*Stachytarpheta jamaicensis*	9	35	10	39	12	46	14	51	15	57
14	*Cordyline fruticosa*	7	28	8	31	9	36	11	40	13	50
15	*Cordyline fruticosa*	10	35	10	38	12	43	13	48	15	54
16	*Excoecariaco chinchinensis*	8	28	9	33	10	38	12	44	13	48
17	*Sidarhom bifolia*	9	30	9	33	10	35	11	40	12	43
18	*Vernonia arborea*	9	33	10	38	11	41	13	49	16	57
19	*Erythrina* sp.	7	25	8	29	9	33	10	36	11	39
20	*Blumea balsamifera*	10	36	11	41	12	45	13	49	15	56
21	*Artocarpus altilis*	7	27	8	31	9	34	10	36	11	40

Remarks: CHP = chloramphenicol; IZ = inhibition zone (in mm); AA=Anticandidal activity (%)

Our investigation into antimicrobial activity against *C. albicans* of several medicinal plants collected from Bentian tribe showed that four plant extracts, i.e. *B. balsamifera*, *T. rhomboidea*, *S. jamaicensis* and *V. arborea* possessed the most activity at low concentration of extract applied. Based on soluble fractions activity, *B. balsamifera* displayed the high possibility to be a source of anticandidal compounds in its polar and non polar fractions. Biological activity of *B. balsamifera* has been reported previously, such as wound healing activity of the essential oil from the leaves, anti hyperglycemic activity of the residues fraction and free-radical scavenging activity of the leaves extract [11-13]. Some flavonoids had been isolated from the leaves extract of the plant [14]. To the best of our knowledge, the antimicrobial activity against *C. albicans* of the plant extract has not been reported.

Candida albicans is thought to be the major fungal pathogen of humans [15]. *C. Albicans* accounts for approximately 50% of cases of candidemia associated with colonization of in dwelling devices, such as catheters, endotracheal tubes, and pacemakers [16,17]. 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 immuno suppressant or anticancer therapy [18, 19]. A limited number of available antifungal drugs and repeated exposure to these limited antifungal agents led to the rapid development of drug resistance [20]. Potent activity of the plant extracts against *C. albicans* suggests the possibility for the treatment of candidemia, nosocomialinfection, and other Candida infection-caused diseases.

TABLE 3. Anticandidal Activity of the Ethyl Acetate Extracts of Medicinal Plants from Bentian Tribe.

No	Samples	Amount of extracts applied									
		25 µg/well		50 µg/well		100 µg/well		200 µg/well		400 µg/well	
		IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)
-	Acetone (control)					0					
-	Chloramphenicol (+)					100					
1	*Macaranga gigantea*	7	29	8	32	9	37	10	40	12	47
2	*Poikilospermum suaveolens*	0	0	0	0	0	0	0	0	9	37
3	*Ceiba pentandra*	0	0	0	0	0	0	0	0	0	0
4	*Ceiba pentandra*	0	0	0	0	0	0	8	33	10	40
5	*Fibraurea tinctoria*	0	0	0	0	0	0	0	0	10	38
6	*Ficus* sp.	0	0	0	0	6	26	9	36	11	44
7	*Gonocaryum* sp.	6	26	8	31	8	34	10	38	11	45
8	*Mallotus mollissimus*	7	26	8	33	9	38	11	45	13	53
9	*Triumfettar homboidea*	7	28	10	38	10	41	11	44	13	49
10	*Cocos nucifera*	0	0	0	0	0	0	0	0	0	0
11	*Vitex pinnata*	0	0	0	0	9	37	10	41	12	46
12	*Scoparia dulcis*	7	30	9	37	10	40	11	43	12	47
13	*Stachytarpheta jamaicensis*	9	37	11	42	11	46	12	52	14	57
14	*Cordyline fruticosa*	0	0	0	0	0	0	0	0	8	33
15	*Cordyline fruticosa*	7	32	10	38	10	43	12	46	12	50
16	*Excoecariaco chinchinensis*	0	0	0	0	8	34	10	42	12	49
17	*Sidarhom bifolia*	0	0	8	32	10	40	10	44	11	48
18	*Vernonia arborea*	0	0	7	28	8	33	10	42	11	45
19	*Erythrina* sp.	9	36	10	41	11	47	12	51	13	54
20	*Blumea balsamifera*	9	39	11	44	11	47	12	52	13	56
21	*Artocarpus altilis*	7	31	8	35	9	39	10	44	12	49

Remarks: CHP = chloramphenicol; IZ = inhibition zone (in mm); AA=Anticandidal activity (%)

TABLE 4. Anticandidal Activity of the Ethanol Extracts of Medicinal Plants from Bentian Tribe.

No	Samples	Amount of extracts applied									
		25 µg/well		50 µg/well		100 µg/well		200 µg/well		400 µg/well	
		IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)	IZ (mm)	AA (%)
-	Acetone (control)					0					
-	Chloramphenicol (+)					100					
1	*Macaranga gigantea*	9	30	11	37	11	38	11	39	11	38
2	*Poikilospermum suaveolens*	0	0	0	0	0	0	0	0	11	37
3	*Ceiba pentandra*	7	23	8	29	11	36	11	39	13	46
4	*Ceiba pentandra*	0	0	0	0	0	0	0	0	0	0
5	*Fibraurea tinctoria*	0	0	0	0	7	25	9	31	12	40
6	*Ficus* sp.	7	25	9	30	10	35	12	39	13	45
7	*Gonocaryum* sp.	0	0	0	0	0	0	7	23	10	34
8	*Mallotus mollissimus*	0	0	6	22	9	31	10	35	12	41
9	*Triumfettar homboidea*	0	0	0	0	0	0	7	25	10	35
10	*Cocos nucifera*	0	0	0	0	7	23	9	31	11	37
11	*Vitex pinnata*	0	0	0	0	0	0	0	0	0	0
12	*Scoparia dulcis*	6	22	8	26	9	32	11	38	12	41
13	*Stachytarpheta jamaicensis*	6	19	7	24	8	28	10	33	11	38
14	*Cordyline fruticosa*	7	23	8	27	9	30	10	35	12	43
15	*Cordyline fruticosa*	6	19	6	22	8	26	9	30	11	36
16	*Excoecariaco chinchinensis*	6	20	6	22	8	26	9	32	11	37
17	*Sidarhom bifolia*	5	19	7	23	8	27	9	32	10	36
18	*Vernonia arborea*	10	35	12	43	14	47	15	51	15	53
19	*Erythrina* sp.	9	30	10	33	10	36	11	38	12	42
20	*Blumea balsamifera*	10	34	12	43	14	50	16	55	17	60
21	*Artocarpus altilis*	9	30	9	32	10	34	11	37	12	41

CONCLUSIONS

The present work has proved *Blumea balsamifera*, *Stachytarpheta jamaicensis* and *Vernonia arborea* were active to exhibit anticandidal activity at low concentration. The results open possibility to use the plants as a source of natural anticandidal agents.

ACKNOWLEDGMENTS

This study was funded by an International Research Collaboration and Scientific Publication Grant from DGHE-Ministry of Research and Technology and Higher Education, Republic of Indonesia (Contract no. 140/SP2H/PL/Dit.Litabmas/II/2015).

REFERENCES

1. I. W. Kusuma, E.T. ArungE. Rosamah, S. Purwatiningsih, H. Kuspradini, Syafrizal, J. Astuti, Y. U. Kim and K. Shimizu, J. Nat Med. **64**, 223-226(2010).
2. G. Brahmachari,"Natural Products in Drug Discovery: Impacts and Opportunities-An Assessment" in *Bioactive Natural Products*, edited by G. Brahmachari (World Scientific, Singapore, 2012), pp. 1-199.
3. E. Pramono."The traditionaluse of traditionalknowledge and medicinalplants in Indonesia"inMulti-Stakeholder Dialoque on Trade, Intellectual Property and Biological Resources in Asia, BRAC Centre for Development Management, Rajendrapur, Bangladesh, 2002.
4. D.J.R. Leaman, Yusuf andH.S. Roemantyo,*Kenyah Dayak Forest Medicines*(World Wide Fund for Nature Indonesia Programme, Jakarta, 1991).
5. I. ChaniagoandS.F. Stephen. Economic Botany **52,**229-250(1998).
6. S. Susiarti,J. Tropical Ethnobiology**1**,52-64(2004).
7. L.F. Fehri, H. Wroblewski and A. Blanchard. Antimicrobial Agents and Chemotherapy **51,** 468-474(2007).
8. N. Fukuyama, M. Shibuya and Y. Orihara. Chem. Pharm. Bull. **60** 377-380(2012).
9. F. Yusro, Y. Mariani, F. Diba and K. Ohtani, Kuroshio Science **8**, 33-38(2014).
10. B. Singh, P.M.Sahu andM.K. Sharma.Phytomedicine**9**, 355-359(2002).
11. Y. Pang, D. Wang, X. Hu, H. Wang, W. Fu, Z. Fan, X. Chen and F. Yu, J. Traditional Chinese Medicine **34**, 716-724(2014)
12. Y. Xia, J. Zuo, X. Li et al. Chinese Herbal Medicines **6**, 136-139 (2014)
13. F. Nessa, Z. Ismail, N. Mohamed and M. R. H. M. Haris, Food Chemistry **88**, 243-252 (2004)
14. D.M.H. Ali, K.C. Wong and P.K. Lim. Fitoterapia**76**, 128-130(2005)
15. J. Karkowska-Kuleta, M. Rapala-Kozik and A. Kozik. Acta Biochim. Pol. **56**, 211-224(2009).
16. E.M. Kojic and R.O. Darouiche. Clin. Microbiol. Rev. **17**, 255-267(2004).
17. G. Ramage, S.P. Saville, D.P. Thomas and J. L. Lopez-Ribot, Eukaryot. Cell **4**, 633-638(2005).
18. G.J. Alangaden. Infect. Dis. Clin. North Am. **25**, 201-225(2011).
19. W.R. Jarvis. Clin. Infect. Dis. **20**, 1526-1530(1995).
20. M.H. Miceli and S.A. Lee. Mycoses **54**, e666-e678(2011).