Available online at www.sciencedirect.com
ScienceDirect
Procedia Environmental Sciences 20 (2014) 772 – 777
Procedia Environmental Sciences

4th International Conference on Sustainable Future for Human Security, SustaiN 2013

The efficacy of the oleic acid isolated from *Cerbera manghas* L. seed against a subterranean termite, *Coptotermes gestroi* Wasmann and a drywood termite, *Cryptotermes cynocephalus* Light

Didi Tarmadi[a]*, S. Khoirul Himmi[a,b], Sulaeman Yusuf[a]

[a]*Research and Development Unit for Biomaterials, Indonesian Institute of Sciences (LIPI), Jl. Raya Bogor KM. 46, Cibinong, Bogor 16911, Indonesia*
[b]*Research Institute for Sustainable Humanosphere (RISH), Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan*

Abstract

The efforts in establishing sustainable pest management drive termite control not only focus on termite extermination, but also concern on human health and environmental protection. Bioactive compounds isolated from Bintaro (*Cerbera manghas*) have been reported to have biological activity against insect such as food deterrent, oviposition and growth inhibitor, and also contact poison. The study on efficacy of oleic acid isolated from *C. manghas* seed extract against subterranean termite *Coptotermes gestroi*and Drywood termite *Cryptotermes cynocephalus* has been thoroughly examined. The extraction and separation method yielded ten fractions, with fraction three (F3) as the highest yield. Chemical compound analysis of F3 by Gas Chromatography and Nuclear magnetic resonance spectroscopy showed oleic acid as chemical compound. The bioassay of oleic acid against *C. gestroi* and *C. cynocephalus* was evaluated by no-choice feeding test and Indonesia National Standard (SNI) 01-7207-2006 respectively. Oleic acid showed low termicidal activity as it delivered low mortality in both species, and generated lower protection against *C. cynocephalus*.

Selection and peer-review under responsibility of the SustaiN conference committee and supported by Kyoto University; (RISH), (OPIR), (GCOE-ARS) and (GSS) as co-hosts

Keyword: *Cerbera mangha*; oleic acid; *Coptotermes gestroi*; *Cryptotermes cynocephalus*

* Corresponding author. Tel.: +62-21-87914511; fax: +62-21-87914509.
*E-mail address:*didi@biomaterial.lipi.go.id

Selection and peer-review under responsibility of the SustaiN conference committee and supported by Kyoto University; (RISH), (OPIR), (GCOE-ARS) and (GSS) as co-hosts
doi:10.1016/j.proenv.2014.03.093

1. Introduction

Asian subterranean termite, *Coptotermes gestroi*, is the major pest in Asia Pacific region, recognized as destructive species and economically important pest [1, 2]. Another important termite species in the region is Indo-Malaya dry wood termite, *Cryptotermes cynocephalus*. Both termite cause serious damage to buildings and structures, estimated more than $400 million per year in Southeast Asia only [3]. Lee et al. [4] reported that *C. gestroi* accounts for more than 85% structural damages on the urban areas in Malaysia, Thailand and Singapore. Thus, the application of termite control is very important to protect building structure from termite infestation. However, most termicides use chemicals identified as Persistent Organic Pollutants (POPs) such as aldrin, coldrane, dieldrin, endrin, etc. [5], and excessive use of synthetic chemicals leads to serious environmental pollution. Rising interest on more environmentally safe pesticide has increased the efforts to find alternatives to the broadly used synthetic chemicals.

The challenges in establishing sustainable termite management focus not only on termite extermination, but also toward human health and environmental protection. Plant extracts have been reported to have repellent and toxic effect against termite [6], and to be promising alternatives for pest control in the future [7]. Researchers are seeking safer treatment, reliable and functional application on preventive and remedial control. Ohmura et al. [7] reported antifeedant activity of some flavonoids and their related compounds against the subterranean termite *Coptotermes formosanus* Shiraki. Himmi et al. [8] also evaluated bioefficacy performance of neem-based formulation on wood protection and soil barrier against *C. gestroi*.

Bintaro (*Cerbera manghas*) belongs to the poisonous Apocynaceae family found in coastal habitats and riverbanks area. Its deadly poisonous seeds contain the potent glycoside compound called cerberin. The extract of *C. manghas* showed high insecticidal activity against *C. gestroi* [9], *Eurema sp*. [10] and *Sitophilus oryzae* [11]. Hashim et al. [12] reported that extracts of *Cerbera odollam* showed the high antifungal activity against *Trametes versicolor*, *Pycnoporus sanguineus*, and *Schizophyllum commune*.

Oleic acid is the major component of triglyceride found in the seed of *C. manghas*. Oleic acid, a monounsaturated fatty acid, has shown activity in cancer prevention, and anticancer effects [13]. Apart from its clinical effect, fatty acid and triglyceride compounds have showed termicidal [8] and larvicidal activity [14]. Due to the previous works on this plant [15, 16], in this study, we evaluated the efficacy of oleic acid isolated from seed extract of *C. manghas* against a subterranean termite, *C. gestroi* and a dry wood termite, *C. cynocephalus*.

2. Materials and Method

2.1. Isolation of Oleic Acid

C. manghas was obtained from Bogor, Indonesia. Seeds of *C. manghas* were sun dried and powdered into 40 mesh. 2000 g seed powder was macerated using methanol, and then the filtrate was collected and separated from residue. The filtrate was evaporated by rotary evaporator (RV 10 Digital, IKA Works GmbH & Co., Germany) at 40 ^{0}C to obtain dried extract. 175 g dried extract was dissolved on 600 ml aquadest and n-hexane (1:1) solution and extracted by separating the funnel. Aquadest fraction was separated and added by 300 ml ethyl acetate (EA) for further extraction.

24 g dried extract of EA fraction was proceeded to column chromatography (100 × 3 cm, Si gel 60–120 mesh, 350 g). Elution was carried out with different proportions of n-hexane and chloroform in the following ratio: (100:0 (4 x 150 ml), 50:1, 25:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:25, 1:50, 0:100) (4 x 150 ml) respectively. Extract fractions were collected and evaluated by thin layer chromatography, and the fractions with the same retention value (R_f) were combined as one fraction. 10 fractions were obtained, and each fraction was analyzed by gas chromatography-mass spectrometry (GCMS-QP2010S, Shimadzu Co. Japan). From all fractions, F3 was the only fraction with pure compound and subjected to ^{1}H-nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR (CDCl3, 500 MHz)(JNM-ECA 500, JEOL Ltd. Japan). F3 was further evaluated for bioassay.

2.2. Bioassay

Subterranean termite, *C. gestroi*, and drywood termite, *C. cynocephalus*, were collected from a laboratory culture at R&D Unit for Biomaterials LIPI, Bogor.

Bioassay against *C. gestroi* was referred to no-choice feeding test, according to Ohmura et al. [7]. A test container was made of a petri dish glass (rim diameter 6cmheight 5cm) with 3 mm hard plaster paris at bottom and moistened with 2 ml of deionized water. Paper discs (diameter13 mm; Whatman International) were permeated with *C. manghas* extract (F3) in various concentrations 1%, 2%, 3%, 4% and 5% (*v/v*). The treatment retention was 1.0% (*w/w*) per disc. The control discs were untreated. The discs were dried at 60~ for 12h followed by drying in a vacuum desiccator for 1 day. Fifty workers and 5 soldiers, and a treated paper disc were entered into a test container. Bioassay was carried out for 14 days. Termite mortality was observed per two days periods and in the final period observation, the mass loss of paper disc was evaluated.

Bioassay against *C. cynocephalus* was conducted according to Indonesia National Standard (SNI 01-7207-2006). Rubber woods (*Hevea brasiliensis*) with dimension 25 (R) x 20 (T) x 50 (L) mm were evenly brushed by *C. manghas* extract (F3) in various concentrations1%, 2%, 3%, 4% and 5% (v/v). Treated woods were air dried for 14 days at room temperature to evaporate solvent. Fifty workers of *C. cynocephalus* were put into glass tube (18 mm diameter and 40 mm height) attached on the surface of treated woods, and top of glass tubes were covered by cotton. The test was carried out for 12 weeks and in the end of test, termite mortality rate and sample weight loss were recorded. Efficacy performance was evaluated according to SNI 01-7207-2006 (Table 1).

Table 1. Efficacy/durability criteria against dry wood termite (SNI 01-7207-2006)

Class	Efficacy/Durability	Weight loss (%)
I	Very High	< 2.0
II	High	2.0 – 4.4
III	Moderate	4.4 – 8.2
IV	Low	8.2 – 28.1
V	Susceptible	> 28.1

3. Results and Discussion

3.1. Identification of Oleic Acid

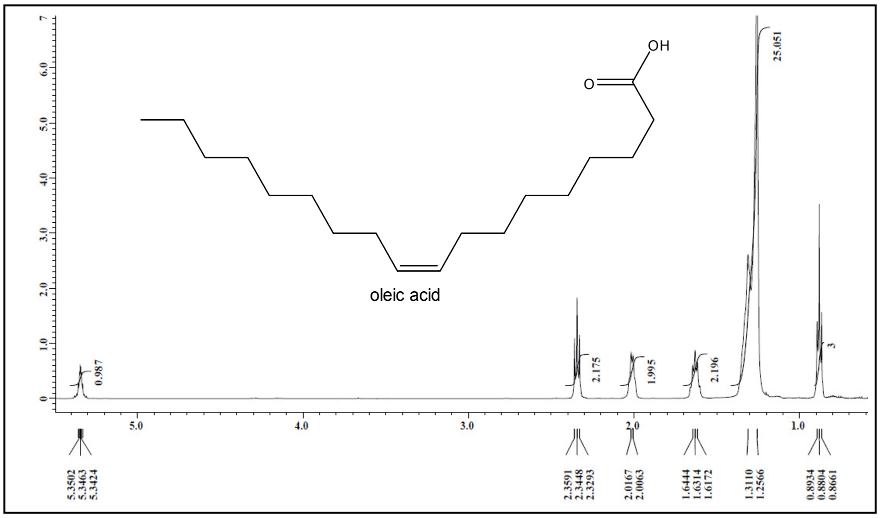

Fig.1 ^{1}H-NMR Spectrum of F3 fraction isolated from *C. mangas* seed.

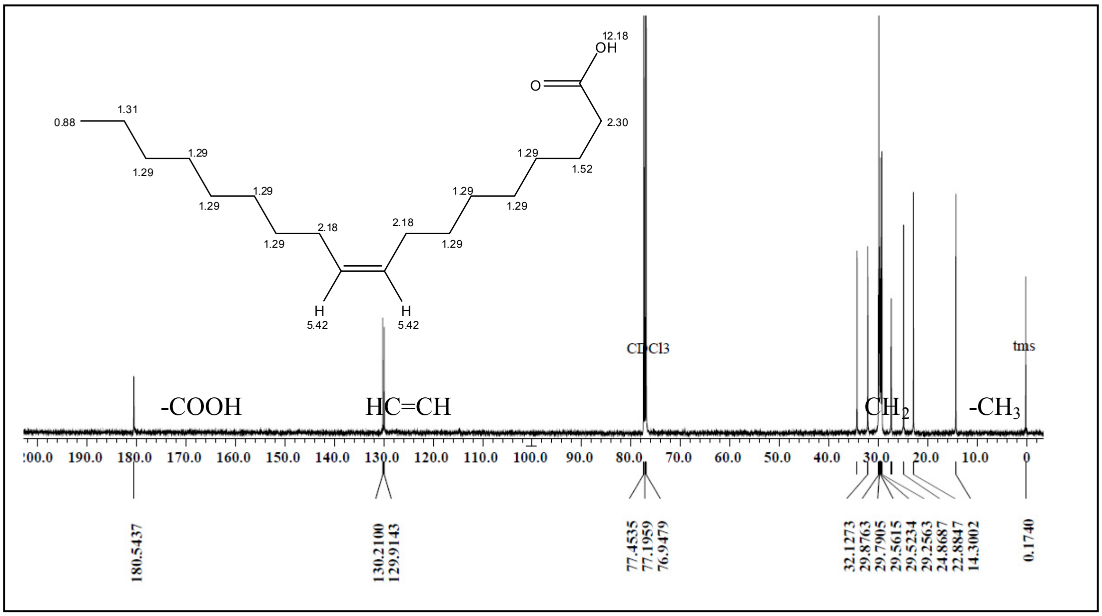

Fig.213C-NMR Spectrum of F3 fraction isolated from *C. mangas* seed.

The extraction of seeds of *C. manghas* yielded 10 fractions, namely F1 (0.218%), F2 (0.289%), F3 (0.950%), F4 (0.301%), F5 (0.389), F6 (0.212%), F7 (0.478%), F8 (0.378%), F9 (0.218%), F10 (0.297%). All those extracts were subjected into GC-MS analysis and only F3 contained a single compound, alleged as oleic acid. The analysis of chemical compound in F3 fraction was carried out by ^{1}H NMR. Fig. 1 shows that spectral data of ^{1}H-NMR from F3. δ_H 0.88 (t, J = 7.2 Hz) indicated the presence of methyl (-CH_3), while long aliphatic chain was indicated by δ_H 1.25-1.31 (24H, 12 x CH_2, bs), 1,63 (2H, CH_2, quintet, J = 7.1 Hz), 2.01 (bd, CH_2, J = 5.4 Hz) and 2.34 (CH_2, t, J = 7.8 Hz). Double bond (HC=CH) was also presented at δ_H 5.35 (m, J = 3.9 Hz).

F3 fraction was further evaluated by ^{13}C-NMR. The spectral data of ^{13}C-NMR is displayed on the fig. 2. δ_C 180.54 indicated the presence of carboxylate functional groups 54 (-COOH), δ_C 130.21 and 129.91 represented double bond (HC=CH), δ_C 14.30 showed methyl group (-CH_3), while long chain aliphatic (CH_2) was suggested by δ_C in between 22.88 to 32.13. The evaluation of both spectral data of ^{1}H-NMR and ^{13}C-NMR, and literature survey suggested that the chemical compound in F3 is oleic acid, $C_{18}H_{34}O_2$ (Molar mass 282.46).

3.2. Efficacy of oleic acid against termite

Table 2 presents the mortality rate of *C. gestroi* after it was subjected into paper disc treated by oleic acid (F3 fraction) in 14 days observation. The data shows that the higher the concentration, the higher the mortality.

Table2. Daily observation of mortality rate of subterranean termite *C. gestroi*

Concentration	Termite mortality rate (%)*						
(%)	2	4	6	8	10	12	14
Untreated	5.33±1.15	9.33±1.15	13.33±3.06	16.00±0	18.00±2.00	18.00±2.00	18.00±2
1	6.67±1.15	10.00±2.00	14.67±1.15	17.33±1.15	18.67±2.31	19.33±1.15	19.33±1.15
2	6.67±1.15	10.67±1.15	14.00±2.00	18.00±2.00	18.67±1.15	19.33±1.15	20.00±0
3	6.67±1.15	11.33±1.15	15.33±3.06	18.67±1.15	19.33±1.15	20.67±2.31	21.33±1.15
4	8.00±2.00	13.33±1.15	19.33±1.15	21.33±1.15	24.67±1.15	26.00±0	30.00±2
5	9.33±1.15	19.33±1.15	21.33±1.15	24.67±2.31	27.33±2.31	30.67±1.15	33.33±1.15

*Values are means ± standard deviations from three replications.

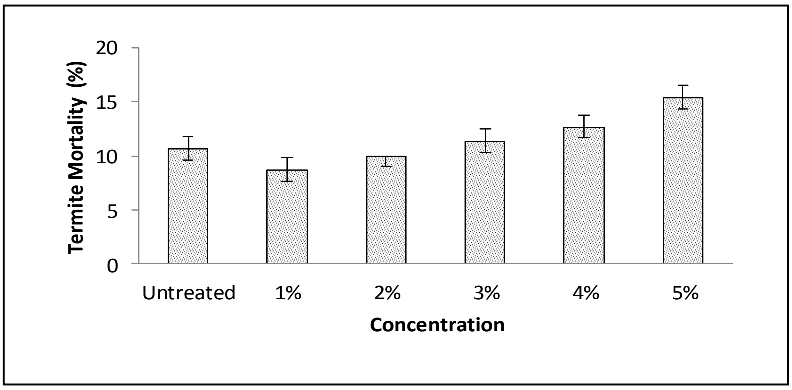

Fig. 3 Mortality rate of drywood termite *C. cynocephalus* after 12 weeks bioassay.

Termite mortality rate was relatively low at concentration below 3%, and the highest concentration only delivered 30% mortality. The results indicate that oleic acid is less toxic against *C. gestroi*, generated from low mortality rate. Weight loss data is displayed on table 3, representing the protection ability of oleic acid against *C. gestroi*. The data suggested that there are no significant differences on protection against termite at concentration 1%, 2% and 3%, generated 49.57%, 49.84%, 48.54% weight loss respectively. The highest concentration of oleic acid (5%) delivered 36.80% weight loss.

Figure 3 displays mortality rate of dry wood termite, *C. cynocephalus* after 12 weeks subjection on wood treated by oleic acid (F3 fraction) at various concentrations. The data indicates low mortality rate, only delivered 15.3% mortality at highest concentration used. Table 3 displays weight loss of the samples against *C. cynocephalus*, and efficacy performance which was evaluated based on classification provided by Indonesia National Standard (SNI). The data shows that at concentration lower than 4%, oleic acid resulted low efficacy against dry wood termite (class IV), while at highest concentration, oleic acid delivered moderate efficacy (Class III) (refer to table 1).

Table3. Sample weight loss after subjected into termite bioassay

Concentration (%)	Weight loss (%)*	
	Subterranean termite *C. gestroi*	Dry wood termite *C. cynocephalus*
Untreated	50.00±2.71 a	9.68±0.05 ab
1	49.57±4.85 a	10.98±0.20 a
2	49.84±7.82 a	9.60±0.14 ab
3	48.54±2.57 a	9.36±0.10 bc
4	42.30±3.02 ab	8.64±0.13 bc
5	36.80±2.92 bc	7.5±0.16 c

*Values are means ± standard deviations from three replications.
Means followed by the same letter within a column are not significantly different (Tukey's test: $P < 0.05$)

Termite mortality rate and sample weight loss are important parameters in evaluating efficacy of a substance against termite. The mortality rate is related to toxicity of a substance, while the weight losses of the samples generate antifeedant activity and protective ability against termite. The results indicated that in pure compound, oleic acid isolated from *C. mangas* seed showed lower toxicity compared to raw methanol extract. Methanol extract of *C. mangas* seed (10%, *v/v*) delivered 100% termite mortality against *C. gestroi*, with 12.77% sample weight loss [11]. The fact that oleic acid treatment was able to deliver higher mortality than untreated sample indicated that oleic acid treatment had effect against termite, but the given mortality rates on *C. gestroi* were considerably low. Weight loss data also suggested that oleic acid showed low antifeedant activity and low protective ability against termite.

Bioassay results against *C. cynocephalus* indicated that oleic acid also showed low toxicity. The mortality rate of *C. cynocephalus* was even lower than *C. gestroi*. Even though concentration treatment of oleic acid showed correlation with mortality rate, as higher concentration delivered higher mortality (Fig. 3), the mortality rate of untreated sample, which was higher than 2% concentration, has to be taken into consideration. Weight loss assessment showed by only one concentration (5%) delivered moderate protection or class III on durability performance.

Rahuman [14] reported that the extract and fraction of *C. colocynthis* that contain oleic and linoleic acid demonstrate a high larval mortality against *A. aegypti* and *C. quinquefasciatus*. Tarmadi et al. [16] also reported high larvicidal activity of raw methanol extract of *C. mangas* seed against *A. aegypti* and *C. quinquefasciatus*. However, oleic acid isolated from *C. mangas* could not replicate its larvicidal performance against termite. In some cases, plant extracts in pure mode deliver lower insecticidal activity. Himmi [8] suggested that pure azadirachtin isolated from neem oil had lower efficacy against termite than neem oil itself. Based on the results, Oleic acid showed low termicidal activity as it provided low mortality in both tested species, and delivered lower protection against *C. cynocephalus* than *C. gestroi*.

References

1. Scheffrahn RH, & Su NY. Asian Subterranean Termite, *Coptotermes gestroi* (=havilandi) (Wasmann) (Insecta: Isoptera: Rhinotermitidae). *University of Florida IFAS Extension (EENY128)* 2000; 1-5.
2. Jenkins TC, Jones SC, Lee CY, Forschler BT, Chen Z,Martinez GL, Gallagher NT, Brown G, Neal M,Thistleton B, Kleinschmidt S. Phylogeography illuminates maternal origins of exotic *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Molecular Phylogenetics and Evolution* 2007;**42**: 612–621.
3. Yeap BK, Othman AS, & Lee CY. Genetic Analysis of Population Structure of *Coptotermes gestroi* (Isoptera: Rhinotermitidae) in Native and Introduced Populations. *Environmental Entomology* 2011;**40**(2): 470-476. doi: 10.1603/en10108
4. Lee CY, C. Vongkaluang, Lenz M. Challenges to subterranean termite managementof multi-genera fau-nas in Southeast Asia and Australia. *Sociobiology* 2007;**50**: 213-221.
5. UNEP/FAO/Global IPM Facility Expert Group on Termite and Biology Management. *Finding Alternatives to Persistent Organic Pollutants (POPs) for Termite Management*. United Nations Environment Programme (UNEP); 2000.
6. Bläske VU, & Hertel H. Repellent and Toxic Effects of Plant Extracts on Subterranean Termites (Isoptera: Rhinotermitidae).*Journal of Economic Entomology*. 2001;**94**(5), 1200-1208. doi: 10.1603/0022-0493-94.5.1200
7. Ohmura W, Shuichi D, Aoyama M, Ohara S. Antifeedant activity of flavonoids and related compounds against the subterranean termite*Coptotermes formosanus* Shiraki. *Journal of Wood Science* 2000;**46**(2), 149-153. doi: 10.1007/BF00777362
8. Himmi SK, Tarmadi D, Ismayati M, Yusuf S. Bioefficacy Performance of Neem-based Formulation on Wood Protection and Soil Barrier against Subterranean Termite, *Coptotermes Gestroi* Wasmann (Isoptera: Rhinotermitidae). *Pro. Env. Sci.*, 2013;**17**: 135-141. doi: 10.1016/j.proenv.2013.02.021
9. Tarmadi D, Ismayati M, Himmi SK, Yusuf S.Antitermite activitiy of *Carbera manghas* L seeds extracts. *Proc. the 7th Pacific Rim Termite Research Group*. Singapore. 2010. p. 28-31.
10. Utami S. Laboratory evaluation on insecticidal activity of Bintaro (*Cerbera odollam* Gaertn) against *Eurema spp*. (*in Indonesian*). *Journal on Forest & Plant Studies* 2010; **7**: 211-220.
11. Tarmadi D, Guswenrivo I, Prianto AH, Yusuf S. The effect of *Cerbera manghas* (Apocynaceae) seed extract against Storage Product Pest *Sitophilus oryzae* (Coleoptera: Curculionidae). *Proc. the 2nd Int. Symp. of Sustainable Humanosphere*. Bandung: 2012.
12. Hashim R, Boon JG, Sulaiman O, Kawamura F, Lee CY. Evaluation of the decay resistance properties of Cerbera odollam extracts and their influence on properties of particleboard. *International Biodeterioration & Biodegradation* 2009;**63**(8): 1013-1017. doi: 10.1016/j.ibiod.2009.07.002
13. Waterman, E., & Lockwood, B. Active Components and Clinical Applications of Olive Oil. *Altern. Med. Rev.*2007;**12**(4): 331-342.
14. Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitol Res* 2008;**103**(6): 1383-1390. doi: 10.1007/s00436-008-1146-6
15. TarmadiD, Prianto AH, Guswenrivo I, Kartika T, Yusuf S. The efficacy of *Carbera odollam* Gaertn and *Brugmansia candida* Pers against subterranean termite *Coptotermes sp.*(*in Indonesian*). *J. Wood sci. tech*. 2007;**5**(1): 38 – 42.
16. Tarmadi D, Himmi SK, Yusuf S. Larvicidal activity of *Cerbera manghas* extract against two mosquito vectors, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Proc. the 3rdInt. Symp. of Sustainable Humanosphere*. Bengkulu: 2013.