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Dari: erwin akkas (winulica@yahoo.co.id)

Kepada: editor.rjpt@gmail.com

Tanggal: Jumat, 8 November 2019 21.04 WITA

Dear
Editor of RJPT
With respect.
we have submitted an article with the title Toxicity Assay of Baccaurea motleyana Mull. Arg. Wood Extracts (Rambai) and Chemical Compounds Evaluation for the Most Active Fraction (attached).
Thank you for your attention

Regards
Erwin

draft article rambai erwin.doc
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Re: submit article

Dari: Research Journal of Pharmacy and Technology Monika Daharwal (editor.rjpt@gmail.com)

Kepada: winulica@yahoo.co.id

Tanggal: Sabtu, 9 November 2019 13.48 WITA

Dear Author,
Thanks for submission of paper. The manuscript no. of submitted paper is RJPT – 8745 /08-11-2019.
The status of paper shall be communicated as soon as possible.
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Editor.

> On Fri, Nov 8, 2019 at 6:34 PM erwin akkas <winulica@yahoo.co.id> wrote:
>
> > Dear
> > Editor of RJPT
> > With respect.
> > we have submitted an article with the title Toxicity Assay of Baccaurea motleyana Mull. Arg. Wood Extracts (R ambai) and Chemical Compounds Evaluation for the Most Active Fraction (attached).
> > Thank you for your attention
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> > Regards
> > Erwin

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To,
Erwin
Organic Chemistry Laboratory of Chemistry
Department of Mulawarman University,
Samarinda Indonesia
email: winulica@yahoo.co.id

Subject - Acceptance Letter-Research Journal of Pharmacy and Technology

Dear Author,

With reference to your article titled **'Toxicity Assay of Baccaurea motleyana Mull. Arg. Wood Extracts (Rambai) and Chemical Compounds Evaluation for the Most Active Fraction'** Author by Erwin, Zenthise Gandi Tonapa, Alimuddin. We wish to bring to your kind notice the following

√ We acknowledge the receipt of the above mentioned article.
√ The above mentioned article(s) has been sent to the reviewer of expert comments
√ The above mentioned article(s) have been accepted for publication in the journal.

The probable date of publication is **Research Journal of Pharmacy and Technology;Vol:13No:11:November-:2020**

Thank you for your interest in Research Journal of Pharmacy and Technology
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Tampilkan pesan asli

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The submitted manuscript is ready for publication in Research Journal of Pharmacy and Technology (RJPT) **Vol. 13 Issue 11, November 2020.**

The gallery proof of the paper is attaching herewith for any necessary correction if required, please convey by E. Mail as in the attached format before **August 21, 2020.**

Thanking you,

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RESEARCH ARTICLE

Toxicity Assay of *Baccaurea motleyana* Mull. Arg. Wood Extracts (Rambai) and Chemical Compounds Evaluation for the Most Active Fraction

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ABSTRACT:

Baccaurea motleyana Müll. Arg. (known locally as Rambai), as an edible fruit plant, is one of the plants native to tropical rain forest of East Kalimantan. This plant is spread in several Asian regions such as Malaysia, Thailand and Indonesia. Traditionally Rambai is used to treat stomach and eye diseases and this plant has anticancer potential. The purpose of this study was to conduct initial screening for bioactivity by the Brine Shrimp Lethality Test (BSLT) method and to determine chemical composition of the most active fraction from the extracts of Rambai woods by the GC-MS analysis. The toxicity test against *Artemia salina* larvae showed that n-hexane, ethyl acetate and methanol fraction has LC_{50} values of 39.62, 11.29 and 661.39 ppm, respectively. Furthermore, the acetate fraction as the most active fraction was further fractionated using flash column chromatography and eight fractions were obtained E1, E2, E3, E4, E5, E6, E7, and E8 with LC_{50} values of 1000 >, 1000 >, 159.52, 138.10, 80.36, 46.06, 47.96 and 72.49 ppm, respectively. The results of GC-MS spectrum characterization of E6 (the most active fraction) showed the presence of alkanes (45.57%), alkenes (27.02%), aromatic compounds (20.90%), fatty acid/fatty acid esters (5.71%), and alcohol (0.78%). Eugenol, 4-ethyl-2-methoxy-phenol, and di-n-octyl phthalate are aromatic compounds that have potential as anticancer drugs.

KEYWORDS: *Baccaurea motleyana*, Rambai, Toxicity, bioactivity, Flash column chromatography, anticancer.

INTRODUCTION:

Plants are actually a source of natural medicines to support healthy human life. The use of plants by the community in traditional medicine has been going on for a long time[1,2]. *Baccaurea* is a fairly large genus of plants. Approximately 43 known species of this genus have spread from India, Indonesia (Borneo, Sumatra, Java) Peninsular, Malaysia, Thailand, the Philippines, to the Pacific island[3]. *Baccaurea motleyana* Müll. Arg, known locally as Rambai is one of the genus *baccuarea* that grows in the tropical rain forests of East Kalimantan.

Traditionally, Rambai is used to treat stomach and eye diseases[4]. In addition, *B. motleyana* fruit can be sold and become additional income for people in Sanggau district and Malawi district, West Kalimantan[5,6].

Previous studies have shown that this plant extract contains phenolic compounds. Phenolic compounds from plants found many that are bioactive and be a good drug candidate based on the nature of its activities[4,7]. Anticancer activity test on *B. motleyana* skin extract potentially inhibit the growth of colon cancer cell line (HT-29)[4]. In the present study we will report the toxicity of wood extracts and their fractions and the chemical composition of the most toxic fraction by GC-MS analysis.

MATERIAL AND METHODS:

Sample collection:

Sample of *B. motleyana* wood was collected from Lubuh Sawah, Mugirejo Village, Samarinda City. The sample was identified in the Plant Anatomy and Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University**.**

Extraction and partitioning:

4000grams of dry wood powder macerated using methanol for 2 times 24 hours. The filtrate obtained was separated by solvent using a rotary evaporator at 40°C

and a pressure of 377 mbar until a crude extract was obtained. The crude extract was redissolved in methanol and then it was partitioned using n-hexane then with ethyl acetate. Then fractionated using column chromatography press performed on the active fraction.

Biological assay:

Bioactivity of crude extract and their fractions are determined using the Brine Shrimp Lethality Test (BSLT) method against *Artemia salina* larvae. 1mg of extract was put in the micro plate, dissolved in a few drops of DMSO then added aqudes to make various variations of the concentration (500, 250, 125, 62, 5, 31, 25, 15, 625 and 7.81ppm). Then 10 shrimp larvae were added into each extract solution. Calculation of LC_{50} was carried out using the SAS program after 24 hours[8,9,10,11].

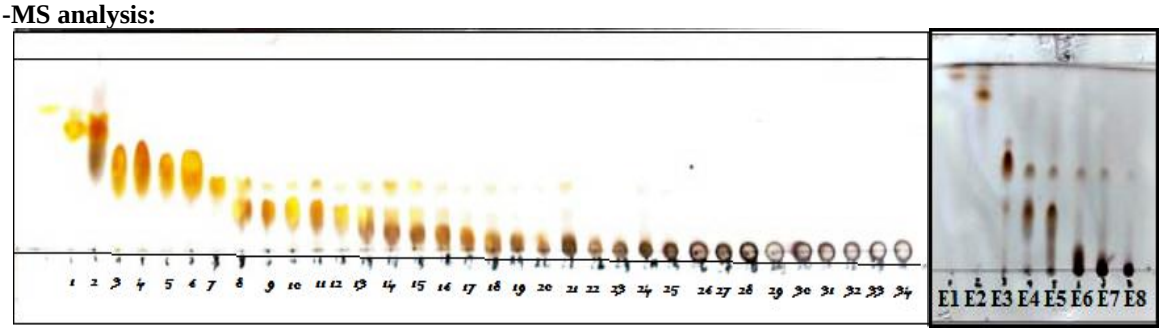
GC-MS analysis:

The most active fraction is characterized its chemical compounds using GCMS QP2010S SHIMADZU, Column: Rtx 5 MS, Column length: 30 meters, ID: 0.25 mm, Film: 0.25um, Carrier gas: Helium, and Ionizing: EI 70 Ev. The compounds will be identified by comparing the NIST data base.

RESULTS AND DISCUSSION:

Extraction and Fractionation:

The crude extract (20grams) was partitioned with n-hexane and ethyl acetate to yield fractions of n-hexane (5.85grams), ethyl acetate (2.99grams) and methanol (0.77grams). Ethyl acetate fraction as the most active fraction (LC_{50}=11.29µg/ml) was further fractionated by flash column chromatography with the gradient polarity elution method to give 34 fractions. The fractions are combined into 8 fractions based on the profile of thin layer chromatography as shown in figure 1.



(a) **(b)**

Fig.1: Chromatogram (a) fractions of flash column chromatography results, (b) The combined fractions flash column chromatography results of the ethyl acetate fraction

Table 1. Weight and LC_{50} values of extract and their fractions

S. No	Extract/Factions	LC_{50} (µg/ml)	Level of toxicity
1	Crude	27.35	Very toxic
2	n-hexane	39.62	toxic
3	Ethyl acetate	11.29	Very toxic
4	Methanol	661.39	Toxic
5	E1	1000 >,	Not toxic
6	E2	1000 >,	Not toxic
7	E 3	159.52	Toxic
8	E 4	138.10	Toxic
9	E 5	80.36	Toxic
10	E 6	46.06	Toxic
11	E 7	47.96	Toxic
12	E 8	72.49	Toxic

Biological assay:

The extract toxicity test againts *Artemia salina* larvae was carried out as a preliminary cytotoxicity assessment tool to determine the potential for activity as an anticancer[8,9]. The results showed that the crude extract, n-hexane fraction, ethyl acetate fraction and methanol fraction obtained LC_{50} values of 27.35, 39.62, 11.29 and 661.39ppm, respectively. Crude extracts and ethyl acetate fractions are very toxic, while n-hexane and methanol fractions are toxic[8]. The results of the toxicity test for the E1-E8 fractions obtained LC_{50} values were 1000 >, 1000 >, 159.52, 138.10, 80.36, 46.06, 47.96, 72.49 ppm, respectively. Only E1da E2 belongs to the non-toxic category while E6 is the most toxic compared to the other fractions as contained in table 1.

GC-MS Analysis:

GC-MS chromatogram of E-6 showed 32 compounds consisting of alkanes, alkenes, fatty acids/fatty acid esters, aromatic and alcohol compounds.

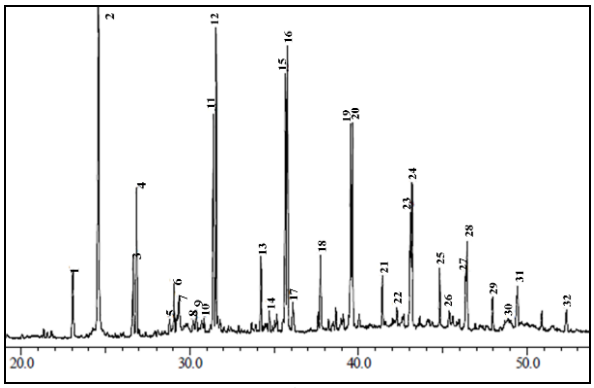


Fig. 2: GC-MS Chromatogram of E6

Table 2. Profile of chemical compounds of E6 based on GC-MS spectrum

Peak	Retention Time (minutes)	Percent Area (%)	Molecular Formula	Molecular Weight	Compound
1	23.059	2.82	$C_{10}H_{12}O_2$	164	*Eugenol*
2	24.590	14.37	$C_{14}H_{22}O_{20}$	206	*3,5-Bis* (1,1-*dimethylethyl*)-*phenol*
3	26.644	2.80	$C_{14}H_{28}$	196	1-*tetradecene*
4	26.840	3.81	$C_{16}H_{34}$	226	*n-tetradecane*
5	28.823	0.74	$C_9H_{12}O_2$	152	4-*ethyl*-2-*methoxy-phenol*
6	29.069	1.46	$C_{11}H_{20}O_2$	184	2-*propenoic acid*
7	29.357	2.15	$C_{11}H_{14}O_3$	194	2,6-*dimethoxy*-4-(2-*propenyl*)-*phenol*
8	30.217	0.75	$C_{15}H_{32}$	212	*n-Pentadecane*
9	30.383	0.99	$C_{16}H_{34}$	226	*5-methyl-Pentadecane*
10	30.710	0.78	$C_{12}H_{26}O$	186	2-*butyl*-1-*octanol*
11	31.398	6.36	$C_{16}H_{32}$	224	*1-Hexadecene/* 1-Cetene
12	31.569	8.69	$C_{17}H_{36}$	240	*n-Heptadecane*
13	34.220	2.10	$C_{11}H_{22}O_2$	186	*Octadecanoid acid, methyl ester*
14	34.720	0.71	$C_{23}H_{28}$	184	*Undecane, 3,7-dimethyl-*
15	35.671	7.78	$C_{18}H_{36}$	252	*1-octadecene*
16	35.808	7.86	$C_{20}H_{42}$	282	*Eicosane*
17	36.119	1.41	$C_{16}H_{33}Cl$	260	1-*chloro-hexadecane*
18	37.741	2.15	$C_{19}H_{36}O_2$	296	11-Octadecenoic acid, methyl ester
19	39.548	6.16	$C_{22}H_{44}$	308	1-*Docosene*
20	39.656	5.00	$C_{16}H_{34}$	226	Isocetane
21	41.426	1.54	$C_{20}H_{42}$	282	Nonadecane, 2-methyl-
22	42.276	0.68	$C_{24}H_{50}$	338	2-*methyl-tricosane*
23	43.093	3.92	$C_{19}H_{38}$	266	9-*Nonadecene*
24	43.186	3.74	$C_{16}H_{34}$	226	*n-Hexadecane*
25	44.826	1.89	$C_{26}H_{54}$	366	n-Hexacosane
26	45.375	0.82	$C_{24}H_{38}O_4$	390	Di-*n-octyl phthalate*
27	46.361	1.72	$C_{14}H_{28}$	196	*Cyclotridecane*
28	46.431	2.12	$C_{18}H_{38}$	254	*n-octadecane*
29	47.962	0.99	$C_{21}H_{44}$	296	Eicosane, 2-mehtyl
30	49.042	0.75	$C_{19}H_{40}$	268	6-*methyl-octadecane*
31	49.445	2.00	$C_{36}H_{74}$	507	Hexatriacontane
32	52.332	0.92	$C_{44}H_{90}$	619	n-Tetratetracontane

The percentage of alkanes group were 45.57% consisting of n-tetradecane (3.81%), n-Pentadecane (0.75%), 5-methyl-Pentadecane (0.99%), n-Heptadecane (8.69%), Undecane, 3.7 -dimethyl- (0.71%), Eicosane (7.86%), 1-chloro-hexadecane (1.41) Isocetane (5%), Nonadecane, 2-methyl- (1.54%), 2-methyl-tricosane (0.68%), n -Hexadecane (3.74%), n-Hexacosane (1.89%), Cyclotridecane (1.72%), n-octadecane (2.12%), Eicosane, 2-mehtyl (0.99%), 6-methyl-octadecane (0.75%), Hexatriacontane (2.00%), n-Tetratetracontane (0.92%). The alkenes group were 27.02% consisting of 1-tetradecene (2.80%), 1-Hexadecene (6.36%), 1-octadecene (7.78%), 1-Docosene (6.16%), 9-Nonadecene (3.92%). 20.90% aromatic compounds consisting of eugenol (2.82%), 3,5-bis (1,1-dimethylethyl) -phenol (14.37%), 4-ethyl-2-methoxy-phenol (0.74%), 2,6-dimethoxy-4- (2-prophenyl) -phenol (2.15%) and di-n-octyl phthalate (0.82%). 5.71% for fatty acids and fatty acid esters consisting of 2-propenoic acid (1.46%), octadecanoid acid, methyl esters (2.10%), 11-octadecenoic acid, methyl esters (2.15%). There is only one alcohol compound, namely 2-butyl-1-octanol (0.78%).

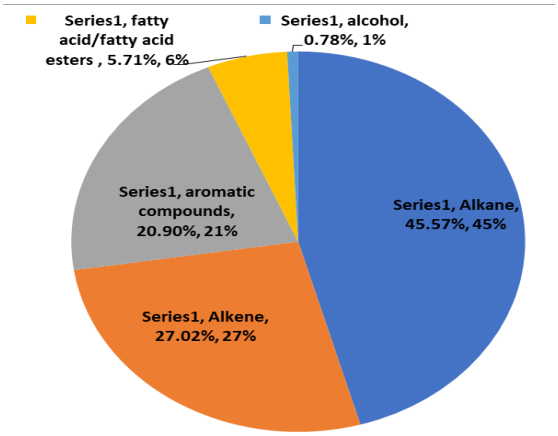


Fig. 3: Diagram percentage phytochemical group identified in E-6.

Many natural aromatic compositions have the potential to be used as medicinal raw materials[12,13,14]. Eugenol (1) can be used to treat or prevent cancer, oxidative stress, inflammation, hyperglycemia, cholesterol, and nerve disorders[15,16]. Eugenol is also used as a tropical analgesic agent used in dental clinic, inhibited aflatoxin production by Aspergillus parasiticus NRRL 2999, and antibacterial activity[17,18,19].

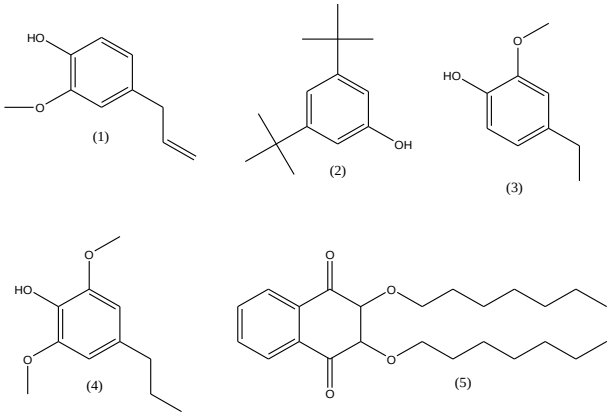


Fig. 4: Aromatic compounds identified in E-6.

3,5-bis (1,1-dimethylethyl) –phenol (2) exhibit weak potency as anti-cancer[20]. However, 4-ethyl-2-methoxy-phenol (3) has antioxidant and anticancer activity[21,22] while 2,6-dimethoxy-4- (2-propenhyl) -phenol (4) has no report on its activities[23]. Bioactivity of di-n-octyl phthalate (5) in Dr. Duke's Phytochemical and Ethnobotanical Databases among others as an antitumor (nasopharynx), and inhibit Production of Tumor-Necrosis-Factor[24].

CONCLUSION:

Based on the results of the study showed that the E6 fraction was the most active fraction with the LC_{50} value 46.06ppm. E6 consists of alkanes (20.9%), aromatic compounds (20.08%), alkenes (17.02%), fatty acids/ fatty acid esters (5.71%), and alcohol (0.78%). Eugenol, 4-ethyl-2-methoxy-phenol and di-n-octyl phthalate are aromatic compounds that have potential as anticancer.

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