Ethnomedicine, phytochemical, and toxicity activity of several alleged medicinal plants from Sebangau National Park, Central Borneo

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Abstract. Indonesia is a country endowed with its abundant biodiversity; and therefore potential in providing future drugs derived from renewable natural materials (e.g. plants). Relevantly, this study aimed to identify and analyze the ethnomedicine, phytochemical, toxicity, and active compounds of several alleged medicinal plant species from Borneo. Results showed local people used those plants to cure various diseases or other human physiological disorders, such as antidote, varices, liver medicine, tonsil medicine, stomachache, diarrhea, sprue medicine, itching, ulcers antihypertension and malaria. Those plants as examined generally contained tannins, saponins, flavonoids, and triterpenoids. Some of them contained alkaloids. Toxicity tests, as represented by LC50 values, exhibited their range from 126.70 to 883.34 ppm. Further, various chemical compounds such as trans-caryophyllene and veridiflorol effectively inflicted a significant effect as antimicrobial, antifungal, and antibiotics. Fatty acids such as hexadecanoic acid, palmitic acid, linoleic acid, octadecadienoic acid, and stearic acid could inhibit the growth of microbes. Phenolic compounds could acts as an antioxidant and antidiabetic. Judging from all the varying LC50 values, ethnobotany-related explorations, and other biomedical/biochemical tests, those analyzed species could be strongly judged as efficacious medicinal plants; and therefore potentially developed as raw material for herbal drugs.

1. Introduction

Medicinal plants can be regarded as one commodity of the potential non-timber forest products (NTFP’s). However, the utilization of such NTFP’s commodity as medicine (drugs) is not yet optimized. There are roughly 20,000 plant species reported that contain active compounds indicatively efficacious to cure particular human diseases; and unfortunately so far only a few of them have traditionally been used for such [1]. Further, [2] mentioned that there are about 1,040 types of forest plants that are beneficial for medicinal purposes in Indonesia. This fact shows that there are quite lot of plants species potentially available to be prospectively developed as medicines (drugs). It therefore leads to the necessity of conducting exploration on the utilization of such potential and prospective medicinal plants. In Borneo, there are eight National Parks, and most of them have served as biosphere reserves for germplasm. One of the benefits of the National Parks is their essential role for research purposes in addition to science, tourism, and recreation endeavors.
The most effective way for the searching and procuring of medicinal plants effectively is through the exploration of ethno botany information. Ethno botany is a science about botany, which studies in-depth regarding the utilization of plants in ethnic customs. Ethno botany studies are not only about the botany’s taxonomic data, but also deal with botanical knowledge of regionalism, and interpretation as well as association reviews. It examines the interrelationships between humans and plants, and scrutinizes the aspects concerning the utilization of such plants preferred for the sake of culture and sustainability of renewable natural resources [3]. Ethno botanists are assigned with tasks of documenting and explaining the complex relationship between culture and the use of plants. It is focused on how plants are used, managed, and perceived in various societies, such as food, medicine, religious practices, cosmetics, dyes, textiles, clothing, construction, tools, currency, literature, belief, rituals, and social life. The use of data associated with traditional medicinal plants derived from ethno botany investigations is an effective way of finding new and useful chemical substances in medicine [4].

In order to know the chemical content of a natural product, it should be done by extraction technique and phytochemical testing. Phytochemical is a science that describes the chemical aspects of a plant. Phytochemical studies include those to describe aspects associated with the variety of organic compounds formed and stored by organisms, their chemical structure, their biosynthesis, their changes and their metabolism, their natural distribution and biological functions, and the isolation and composition of chemical compounds in various plant species [5].

Relevant with such, the research was conducted that took place in Sebangau National Park, in Baun Bango Resort, Baun Bango Village, Katingan Regency, Central Borneo Province. Sebangau area owns its main ecosystem where the peat ecosystem could favorably take place. Historically, this area is a former production forest where there were 13 concessions operating during the period of 1980-1995. Sebangau reserved as National Park is based on the Decree issued by the Minister of Forestry, No: SK.423 / Menhut-II / 2004 dated on October 19, 2004. Inherently, the main purpose of conducting the research there was to examine in-depth whether the particular plant species indigenous from Sebangau Park are efficacious for medicinal uses, whereby such undertaking started from consecutively their ethno botany studies, phytochemical tests, bioassays, isolation of active compounds, until the clinical testing; and the results are forthcoming.

2. Materials and Methods

2.1. Materials
The samples were obtained from Sebangau National Parks, administratively under the Baun Bango Resort, Katingan Regency, in Central Borneo.

2.2. Methods

2.2.1. Material preparation
The research materials included the plants or their tree parts that were presumed or alleged efficacious as medicine (i.e. bark, fruit, leaves, and roots); and then several pieces from those were taken as the representative material samples. Each of the samples was collected, which weighed approximately two kg. The sample was dried in the oven at temperature of 50°C.

2.2.2. Ethnomedicinal study
The study was conducted through in-depth interviews with the communities (local villagers) around the forest regarding the plant species, which they alleged efficacious as medicinal plants; and how they utilized those medicinal plants from the forest.
2.2.3. Extraction
There were three steps in finding out and obtain the crude extract from the materials. Firstly, the material samples after being dried were ground to powder using a milling machine; and then the resulting powder was strained using a multistage screen to obtain the strained powder with a specific dimension (i.e. 40-60 mesh in size). Secondly, the obtained 40-60 mesh-sized powder was extracted with 96% methanol applying the so-called maceration technique at room temperature for 4 times 24 hour; and afterwards the migrated filtrate solution that contained the extract and solvent (methanol) was collected. Finally, the methanol extracts were obtained by separating them each from the filtrate solution using a rotary vacuum evaporator at 54°C (in the ultimate dried solid form).

2.2.4. Phytochemical testing
Phytochemical testing was carried out on the dried methanol extracts to identify the extract constituents using standard procedures. The constituents (compounds) which were tested included those as follows: flavonoid, tannin, saponin, triterpenoid, steriod, hydroquinone, and alkaloids [5].

2.2.5. Toxicity testing
Brine shrimp lethality test (BSLT) signifies as one of the methods for detecting bioactive compounds present in natural materials (including the methanol extracts as such from the particular plant parts/portions) using the organisms of shrimp (*Artemia salina*) larvae. The toxicity properties inflicted by those materials can be known and figured out, based on the number of dead larvae or larva mortality. The solid methanol extracts (as previously obtained using rotary vacuum evaporator) were then prepared in aqueous solution form with concentrations of 5000 ppm by dissolving each into the distilled water, and afterwards diluted further also with distilled water to consecutively 500, 100, 50, and ultimately 10 ppm. If the extract was not soluble in water, dimethylsulphoxide (DMSO) should be added. Further, the seawater in the amount of 400 mL was prepared; and then as much as 600 μL of seawater was taken. Into the 600 μL-seawater was then added as many as 10 shrimp larvae, and then also added 1 mL of aqueous methanol extract (with varying concentrations of consecutively 500, 100, 50, and 10 ppm). Further, the mixture of 600 μL-seawater (that contained 10 shrimp larvae) and 1 mL-methanol extract was then put into the multiwell. Afterwards, the multiwell was covered with thin aluminum sheet and incubated for 24 hours. In each concentration of the extract could then be calculated the average percentage (%) of dead shrimp larvae (larva mortality).

A chemical substance is said to be toxic to the shrimp (*Artemia salina*) larvae, if it affords LC$_{50}$ (lethal concentration for 50% mortality of the shrimp) value less than (<) 1000 μg/ml (ppm) (Meyer, 1982). For the case of methanol extract, the LC$_{50}$ value in this regard implied its lethal concentration that could reach value < 1000 ppm, at which it was able to cause as much 50% mortality as the dead shrimp larvae; and therefore such concentration regarded as toxic.

2.2.6. Analysis of chemical compounds
Methanol extracts from those several presumed (alleged) medicinal plant species were used as material for the analysis of their chemical compounds. The analysis used a Gas Chromatography Mass Spectra (GCMS) device, located at the Forensic Laboratory Center (*Puslabfor POLRI*), Police Headquarters. The GCMS specification is an electron ionization (EI) detector attached on the GC-17A gas chromatograph (Shimadzu), which was combined with MSQP5050A mass spectrometer and the database Wiley 7N2008. The GC/MS instrument (Shimadzu QP2010) run time was set at 39.67 minutes, the initial GC oven temperature at 70°C, final temperature at 290°C, and used DB5MS detector.

3. Results and Discussion

3.1. Ethnomedicine
Ethnomedicine regarding the information about particular plants, strongly believed as efficacious by
local communities (villagers) for medicinal purposes, is often used as a guide in the chemical screening work to develop new drugs. As such, there are 10 (ten) of the important plant species which were found on the research area. The details of those 10 species, particularly about kinds of their ethnomedicinal uses are summarized in Table 1.

Table 1. The plant species prevalently used by local communities for ethnomedicinal purposes

| No. | Species                          | Parts of the plants used | Kinds of medicinal uses/purposes          |
|-----|---------------------------------|--------------------------|------------------------------------------|
| 1   | Kenanga                         | Barks                    | Antidote                                 |
| 2   | Rambai Kuwung                   | Leaves                   | Varices                                  |
| 3   | Akar kuning daun kecil          | Roots                    | Liver medicine                           |
| 4   | Bangang                         | Leaves                   | Liver medicine                           |
| 5   | Kaloan                          | Barks                    | Tonsil medicine                          |
| 6   | Kajak                           | Barks                    | Stomachache, diarea                      |
| 7   | Tapisi                          | Leaves and roots         | Sprue medicine                           |
| 8   | Puri                            | Leaves                   | Itching, ulcers                          |
| 9   | Belawan                         | Barks                    | Antihypertension                         |
| 10  | Pasak bumi                      | Roots                    | Malaria medicine, kidney                 |

*Akar kuning* and *pasak bumi* species are the most widely used plant species by villagers. They believe and allege that those two species afford wide benefits in maintaining human health. Such belief and allegation bring about positive effect on the existence (survival) and sustainability of those species in the forest area.

People (local villagers) surrounding the research study site are still dependent much on the medicinal forest plants, although the synthetic drugs have begun massively entering their living vicinity, which were easier to find. Ethnomedicine information on such medicinal forest plants is often used not only to guide chemical screening work in developing new drugs, but also beneficial in order to preserve (conserve) or sustain the forest itself [6]. On the other hand, there is an indication that there are a lot of challenges faced by particularly ethno botanists that should be overcome in documenting medicinal plants and exploring other species [7].

The identification results on botanical/scientific names for each of the 10 plant species which match any of their already known local/vernacular names (Table 1) are shown in Table 2. Such identification is based on the guidance provided by the Herbarium Bogoriensis Institute, located in Cibinong. Those 10 plant species were each collected from ten different families (Table 2).

Table 2. Botanical name of 10 medicinal plant species

| No. | Species/Local names *) | Botanical (Scientific) names | Family                |
|-----|------------------------|------------------------------|-----------------------|
| 1   | Kenanga                | *Cananga odorata* (Lam) Hook.f. & Thomson | Annonaceae            |
| 2   | Rambai Kuwung          | *Ziziphus horsfieldii* Miq. | Rhamnaceae            |
| 3   | Akar kuning daun kecil | *Arcangelisia flavana* (L.) Merr. | Menispermaceae        |
| 4   | Bangang                | *Tabernaemontana sphaerocarpa* Blume | Apocynaceae           |
| 5   | Kaloan                 | *Vitex pinnata* L.          | Lamiaceae             |
| 6   | Kajak                  | *Dillenia excelsa* (Jack) Martelli ex Gilg. | Dilleniaceae         |
| 7   | Tapisi                 | *Elaeocarpus glaber* Blume | Elaeocarpaceae        |
| 8   | Puri                   | *Mitragyna speciosa* (Korth.) Havil. | Rubiaceae            |
| 9   | Belawan                | *Tristaniopsis obovata* (Benn.) Peter G. Wilson & J.T. Waterh. | Myrtaceae            |
| 10  | Pasak bumi             | *Eurycoma longifolia* Jack | Simaroubaceae         |

*) Please refer to Table 1
3.2. Phytocemical testing

Phytocemical testing results for the methanol extracts originated from particular parts of the ten alleged medicinal plant species (Tables 1 and 2) are disclosed in Table 3. Results showed that those medicinal plants generally contained tannins, saponins, flavonoids, triterpenoids, and alkaloids. All of those extracts exhibited positive test on the presence of tannin compounds. Tannin belongs to the phenolic groups. Tannins are widely present in vascular plants, especially in wooden tissue, in addition to their abundant presence in the leaves. Plants that contain the tannin compounds a lot are generally avoided or disliked by plant-eating animals, because those compounds inflict a sense of splint; and therefore are considered as animal repellents [5].

Cananga odorata, Arcangelisia flava, Tabernaemontana sphaerocarpa, Vitex pinnata, and Eurycoma longifolia revealed the presence of alkaloids (Tables 2 and 3). Alkaloids affords important ecochemical functions in the defense of the plant against pathogenic organisms and herbivores or, as in the case of pyrrolizidine alkaloids, as pro-toxins for insects, which further modify the alkaloids and then incorporate them into their own defense secretions[8].

Alkaloids are pharmacologically active nitrogen-containing compounds; in that they can affect the human central nervous system, reduce appetite, and act as diuretic. Recent reviews disclosed that certain alkaloids are medicinally applied as local anesthetic, stimulants, psychedelics, analgesics, anti-cancer drugs anti-arrhythmia, anti-asthma, and anti-malaria [9].

Table 3. Pyhtocemical testing on chemical compounds in 10 alleged medicinal plant species

| No | Species                                                                 | Compounds | Alkaloid |
|----|-------------------------------------------------------------------------|-----------|----------|
|    |                                                                         | T | S | F | S/T | D | M | W | H |
| 1  | Cananga odorata (Lam) Hook.f. & Thomson                                  | + | - | - | +T  | - | + | + | - |
| 2  | Ziziphus horsfieldii Miq.                                                | + | + | + | +S  | - | - | - | - |
| 3  | Arcangelisia flava (L.) Merr.                                           | + | - | - | +S  | + | - | + | - |
|    |                                                                         |           | /T |
| 4  | Tabernaemontana sphaerocarpa Blume                                      | + | - | - | - | - | - | - | - |
| 5  | Vitex pinnata L.                                                        | + | + | - | +T  | - | - | - | - |
| 6  | Dillenia excelsa (Jack) Martelli ex Gilg.                               | + | + | - | +T  | - | - | - | - |
| 7  | Elaeocarpus glaber Blume                                                | + | - | - | - | - | - | - | - |
| 8  | Mitragyna speciosa (Korth.) Havil.                                      | + | + | - | - | - | - | - | - |
| 9  | Tristaniopsis obovata (Benn.) Peter G. Wilson & J.T. Waterh.             | + | + | - | +T  | - | - | - | - |
| 10 | Eurycoma longifolia Jack                                                | + | - | + | +T  | - | - | + | - |

Remarks: T=tannin; S=Saponin; F=Flavonoid; S/T=Steroid/Triterpenoid; D=Dragendorf; M=Meyer; W=Wagner; H=Hydroquinone; + = positively present (detected); - = negatively present (undetected)

3.3. Toxicity

The BSLT (brine shrimp lethality test) method in this regard as described before is to assess the effectiveness or toxicity of the plant’s methanol extract directed to inflict mortality on the shrimp (Artemia salina L.) larvae [10], [11]. The BSLT results are expressed quantitatively in the calculated LC50 (lethal concentration) values each in this regard also for any of the particular methanol extracts at their various concentrations (ppm) (Table 4). The LC50 values also as described before imply the quantity of chemical dosages or concentrations of a toxic substance (e.g. methanol extract) that could cause as much half death portion (50% mortality) as the dead shrimp larvae; and further a substance with LC50<1000 ppm can be considered as containing active toxic compounds[12]. Results of the toxicity testing (LC50 values) on the methanol extracts from the 10 alleged medicinal plant species are presented in Table 4.
Results strongly indicate that all the alleged medicinal plants species which were investigated exhibited their toxic properties, as their overall LC$_{50}$ values reached below 1000 ppm (Table 4). This implies that all those medicinal plants contained potentially active toxic compounds. The information regarding such toxicity is essential, as in many cases no definite dosages or quantity of drugs are convincingly prescribed thereby often resulting in the drug’s over-dosed prescription and hence worsening human health [13].

Table 4. Toxicity testing results on the methanol extracts from the 10 alleged medicinal plant species

| No | Species                                             | Toxicity (LC$_{50}$ values), ppm |
|-----|-----------------------------------------------------|----------------------------------|
| 1   | *Cananga odorata* (Lam) Hook.f. & Thomson          | 651.489                          |
| 2   | *Ziziphus horsfieldii* Miq.                        | 345.037                          |
| 3   | *Arcangelisia flava* (L.) Merr.                    | 500.704                          |
| 4   | *Tabernaemontana sphaerocarpa* Blume               | 883.339                          |
| 5   | *Vitex pinnata* L.                                 | 851.389                          |
| 6   | *Dillenia excelsa* (Jack) Martelli ex Gilg.        | 427.982                          |
| 7   | *Elaeocarpus glaber* Blume                         | 746.297                          |
| 8   | *Mitragyna speciosa* (Korth.) Havil.               | 650.041                          |
| 9   | *Tristaniopsis obovata* (Benn.) Peter G. Wilson & J.T. Waterh. | 353.566 |
| 10  | *Eurycoma longifolia* Jack                         | 126.696                          |

LC$_{50}$ values = the lethal concentrations of methanol extract that in this regard could reach value < 1000 μg/mL (ppm), at which such extract was able to cause as much 50% mortality as the dead shrimp larvae

In order to develop efficacious medicinal products, it is essential to know or have a reliable data/information about their active and/or toxic constituents (compounds). Unfortunately, little is known about such data/information; and therefore this situation deserves immediate and urgent overcoming actions. In many countries, including the US, herbal medicines are not subjected to or imposed with the same regulatory standards as orthodox (conventional) drugs in terms of efficacy and safety [14]. Accordingly, this raises concerns on their safety and implications for their use as medicines. Toxicity testing can expectedly overcome or at least minimize some of the risks that might occur due to the use of such herbal drugs, thereby avoiding or lowering their harmful effect when used as medicine [15].

3.4. Chemical compounds

Chemical compounds as analyzed in the 10 alleged medicinal plant species and the results are shown in Table 5. Trans-caryophyllene and veridiflorol compounds as present belong to a sesquiterpene group, which could have considerable effects as antimicrobial, antifungal and antibiotic. Fatty acids also as present such as hexadecanoic acid, octadecanoic acid and oleic acid are known able to act as antimicrobial [16].
Table 5. Chemical compounds present in the ten alleged medicinal plant species

| No | Species | Chemical compounds | % |
|----|---------|---------------------|---|
| 1  | Cananga odorata (Lam) Hook.f. & Thomson | trans-Caryophyllene | 13.15 |
|    |         | VERIDIFLOROL | 13.59 |
|    |         | LEVOGLUCOSAN | 8.10 |
|    |         | Z3,Z6,E8-dodecatrien-1-ol | 8.07 |
|    |         | 5-Hepten-1-ol, 2-ethenyl-6-methyl- (CAS) 5-Hepten-1-ol, 6-methyl-2-vinyl- (CAS) | 6.70 |
| 2  | Ziziphus horsfieldii Miq. | Hexadecanoic acid (CAS) Palmitic acid | 18.24 |
|    |         | 9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E) (CAS) | 17.70 |
|    |         | 9,12-Octadecadienoic acid (Z,Z)- (CAS) linoleic acid | 12.01 |
|    |         | Octadecanoic acid (CAS) Stearic acid | 5.73 |
|    |         | Ergosta-5,7,22-trien-3-ol, (3.beta.,22E)- (CAS) Ergosterol | 4.19 |
| 3  | Arcangelisia flava (L.) Merr | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | 40.42 |
|    |         | 3,6-Octadecadienoic acid, methylster (CAS) METHYL 3,6-OCTADECADIENOATE | 24.88 |
|    |         | Hexadecanoic acid (CAS) Palmitic acid | 6.54 |
|    |         | 9-Octadecenoic acid (Z)-, methylster (CAS) Methyloleate | 4.56 |
|    |         | Phenol, 2-methoxy- (CAS) Guaiacol | 3.19 |
| 4  | Tabernaemontanaeae rocarpa Blume | alpha.-Amyrenyl acetate | 13.92 |
|    |         | 9-Octadecenoic acid (Z)- (CAS) Oleic acid | 8.37 |
|    |         | METHYL COMMATE D | 7.50 |
|    |         | Hexadecanoic acid (CAS) Palmitic acid | 5.95 |
|    |         | beta.-Amyrene | 3.97 |
| 5  | Vitex pinnata L. (LEVOGLUCOSAN) | Benzoic acid, 3-hydroxy- (CAS) 3-hydroxybenzoic acid | 11.63 |
|    |         | Phenol, 3,4,5-trimethoxy- (CAS) Anitral | 10.67 |
|    |         | alpha.-Amyrenyl acetate | 9.95 |
|    |         | Phenol (CAS) Izal | 7.38 |
| 6  | Dillenia excelsa (Jack) Martelli ex Gilg. | 1,2,3-Benzenetriol (CAS) 1,2,3-trihydroxybenzene | 34.40 |
|    |         | 1,2-Benzenediol (CAS) Pyrocatechol | 10.89 |
|    |         | Hexadecanoic acid (CAS) Palmitic acid | 10.40 |
|    |         | 9,12-Octadecadienoic acid (Z,Z)- (CAS) linoleic acid | 10.32 |
|    |         | Nonanoic acid (CAS) Nonanoic acid | 3.68 |
| 7  | Elaeocarpus glaber Blume | 9,12-Octadecadienoyl chloride | 23.37 |
|    |         | Hexadecanoic acid (CAS) Palmitic acid | 12.23 |
|    |         | Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol | 9.95 |
|    |         | Hexadecanoic acid (CAS) Palmitic acid | 6.43 |
|    |         | 1,2,3-Benzenetriol (CAS) 1,2,3-Trihydroxybenzene | 5.65 |
| 8  | Mitragyna speciosa (Korth.) Havil. | Hexadecanoic acid (CAS) Palmitic acid | 17.39 |
|    |         | 9,12,15-Octadecatrien-1-ol (CAS) OCTADEC-9,12,15-TRIEN-1-OL | 10.02 |
|    |         | 9-Octadecenoic acid (CAS) Stearic acid | 4.77 |
|    |         | Tetradecal (CAS) Myristaldehyde | 3.65 |
|    |         | EVOGLUCOSAN | 2.99 |
| 9  | Tristaniopsis obovata (Benn.) Peter G. Wilson & J.T. Waterh. | 1,2,3-Benzenetriol (CAS) 1,2,3-trihydroxybenzene | 79.38 |
|    |         | METHYL COMMATE B | 2.97 |
|    |         | LEVOGLUCOSAN | 2.26 |
|    |         | 1,2-Benzenediol (CAS) Pyrocatechol | 2.04 |
|    |         | ALPHA-TOCOPHEROL-ACETAT | 1.62 |
| 10 | Eurycoma longifolia Jack | HEPTADECENE-(8)-CARBONIC ACID-(1) | 15.29 |
|    |         | Hexadecanoic acid (CAS) Palmitic acid | 9.25 |
|    |         | 3,4-DIAZAFLUORANTHEN-2(3H)-ONE | 6.57 |
|    |         | Phenol, 4-methoxy- (CAS) Hqmm | 6.06 |
|    |         | 2H-1-Benzopyran-2-one, 7-hydroxy-6-methoxy- (CAS) Scopoletin | 5.87 |
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