Identification on chemical organic compounds of
Macaranga hypoleuca by using Py-GCMS

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Abstract. Organic chemical compounds of Macaranga spp. have been identified and their bioactive potential have been explored, but Macaranga hypoleuca information was limited. M. hypoleuca includes fast growing and pioneer’s species in secondary forest successions. Limited utilization of M. hypoleuca make this species classified into less well-known species. Present study was conducted to identify the chemical compounds of M. hypoleuca by using Pyrolysis Gas Chromatography-Mass Spectrometry (Py-GCMS QP2010). Samples were originated from stem, bark, sap and leaves of M. hypoleuca. This study was able to identify the compounds of dibromo chloropropane, methoxy 2-propenal, ammonium carbamate, levoglucosan, teraxeron, n-tetra terakseron, hydroxy-2-propenal, carbinol and pentanoic acid as the dominant organic compounds in M. hypoleuca. Further research is needed to isolate and biological activity.

1. Introduction

Macaranga hypoleuca is one of the fast-growing Euphorbiaceae families and can grow well in peat swamp secondary forest [1,2]. M. hypoleuca often used for the succession of former mine reclamation lands or land ex-fire. Characteristics M. hypoleuca which can grow on critical land, with low fertility, makes M. hypoleuca classified into the pioneer plant [3]. Because pioneer plants are resistant to pests and diseases, it is suspected that M. hypoleuca has natural defences that may be of benefit to human. This species is found in many forest areas in Sumatra, but has not been widely used, furthermore, it is a less well-known wood species.

A review of Macaranga genus to determine the benefits of phytochemical and pharmacological has been done, but types M. hypoleuca was not included in the study [4]. The investigation several types of organic chemical compounds from the genus ficus, artocarpus and macaranga to find and development of natural medicines [5]. Evaluation of a crude extract involves Neutral Red (NR) assay, staining Acridine Orange/Ethidium Bromide (AO/EB) and cell cycle analysis to determine anty-cancer activity. The study identified the presence of new organic chemical compounds in the leaves M. hypoleuca namely: flavonoid glycosides, aglycone flavonoids and triterpenoid compound.

This paper intends to provide scientific information on organic chemical compounds of M. hypoleuca from the stem, bark, sap and leaves. Thus, dominant compounds in this study were confirmed through literature studies. The limitation of this study is the identification of chemical compounds M. hypoleuca from South Sumatra habitat.
2. Materials and Methods

2.1. Sample origin
The research sample was taken from the working area of Lakitan Forest Management Unit (KPHP Lakitan) Bukit Cogong. Geographical location is between 102°46'12" to 103°15'36" East Longitude and 02°45'00" to 03°16'48" South Latitude. The KPHP area is located in the Musi Rawas Regency area, South Sumatra Province.

2.2. Material preparation
The technique of sample preparation comply with another place [1], with some modifications. The research material was White Mahang (Macaranga hypoleuca), which are often found in the ex-fire area of the KPHP Lakit Bukit Cogong area. Material taken from forest stand between 10-30 cm in diameter: includes stem, bark, sap and leaves. The bark is peeled from stem and air dried until 20-30% moisture content. The sap and leaves are air dried to a moisture content of 12%. All material is crushed into a powder [6,7] measuring about 20 mesh.

2.3. Py-GCMS analysis
Py-Gas Chromatography-Mass Spectrometry (Py-GCMS) was used to identified chemical organic compounds. Approximately 50 milligrams of sample of material without pre-treatment were analysed using Py-GCMS Shimadzu Pyrolysis Type QP2010 [6] with several different setting. In this study, a high purity helium carrier gas was used with a velocity of 23.7 cm sec\(^{-1}\). Column temperature at 280ºC while pyrolizer temperature of 400ºC. A pressure of 101.0 kPa for 50 minutes.

3. Results and Discussion
Py-CMS analysis of sample show that there are 100 organic chemical compounds derived from leaves (40), sap (15), stem (20) and 25 organic chemical compounds from bark (Figure 1). The organic chemical compounds with highest relative concentration were dibromo chloropropane (DBCP) of 59.84% in sap powder, methoxy 2-propenal (35.69%, stem), ammonium carbamate (26.64%, bark), DBCP (22.04%, leaves), methoxy 2-propenal (19.49%, bark), levoglucosan (12.24%, leaves), taraxerone (11.71%, latex), n- tetra taraxerone (11.55%, latex), hydroxy-2-propenal (11.55%, wood), carbinol (10.25%, wood) and pentanoic acid (8.71%, bark) as listed in Table 1.

| Sample  | Chemical organic               | Retention (min) | % relative | Masa Mol. (g mol\(^{-1}\)) |
|---------|--------------------------------|----------------|------------|--------------------------|
| Sap     | DBCP, C\(_3\)H\(_3\)Br\(_2\)Cl | 3.451          | 59.84      | 234.315                 |
| Stem    | Metoksi 2-propenal, C\(_{10}\)H\(_{16}\)O\(_3\) | 19.750         | 35.69      | 178.187                 |
| Bark    | Ammonium carbamate, CH\(_3\)N\(_2\)O\(_2\) | 3.501          | 26.64      | 78.071                  |
| Leaves  | DBCP, C\(_3\)H\(_3\)Br\(_2\)Cl | 3.444          | 22.04      | 234.315                 |
| Bark    | Metoksi 2-propenal, C\(_{10}\)H\(_{16}\)O\(_3\) | 19.776         | 19.49      | 178.187                 |
| Leaves  | Levoglucosan, C\(_2\)H\(_{10}\)O\(_3\) | 18.159         | 12.24      | 162.141                 |
| Sap     | Tarakseron, C\(_{30}\)H\(_{45}\)O | 49.523         | 11.71      | 424.713                 |
| Sap     | n-tetra tarakseron, C\(_{44}\)H\(_{90}\) | 36.154         | 11.55      | 619.204                 |
| Stem    | Hidroksi 2-propenal, C\(_{11}\)H\(_{12}\)O\(_4\) | 21.440         | 11.55      | 208.213                 |
| Stem    | Cyclopropyl carbinol (CPMO), C\(_3\)H\(_4\)O | 13.970         | 10.25      | 72.107                  |
| Bark    | Pentanoic acid, C\(_3\)H\(_{14}\)O\(_3\) | 17.223         | 8.71       | 146.186                 |
Figure 1. Py-GCMS chromatogram of the organic chemical compound *M. hypoleuca*: (a) leaf, (b) sap, (c) stem, (d) bark.

DBCP is widely used in pesticides against pests and diseases in agricultural crops [8]. DBCP reported altering sperm concentration [8-10]. The impact of pesticides containing DBCP on reducing the reproductive function of men exposed to next 3-6 years have been well reported [8]. DBCP exposure cases in India have been pollute waters and cause cancer [11]. DBCP content in *M. hypoleuca* have function as a natural pesticide except for *Crematogaster spp.* [12,13] and elephant [14]. Since 1979, the use of DBCP in agricultural has been banned, in fact it has been strictly restricted to general uses. The specific cases described above have made it very clear that DBCP has a very high toxicity. Interestingly,
DBCP was present in the leaves and sap of the study samples. The authors suspect that DBCP content is a natural defence from pests and diseases. In fact, *M. hypoleuca* is one of the dominant plants in succession forest stands.

Low mass of methoxy phenol compounds, coniferyl aldehydes (CA, C_{10}H_{10}O_{3}), contained in stem of 35.69%. CA is a natural antimicrobial to prevent candida strains. CA was effective against 65 types of candida fungi [15]. CA is stated to increase protein stability to reduce normal cell damage due to irradiation therapy. CA has the potential to induce Heat Shock Factor-1 (HSF-1) to protect normal eukaryotic cell damage post chemotherapy or radiological intervention [16]. CA has no effect on the reduction of cancer nodules, but protects normal lung cells from the effects of therapeutic irradiation. The protection of normal cells by CA is carried out through a phosphorylation mechanism, namely the addition of a phosphate group to certain proteins [17]. However, among the results mentioned above, CA proved to be effective against fungi, especially candida strains.

Bark contains a carbamic acid functional group, ammonium carbamate (CH_{6}N_{2}O_{2}), with a relative concentration of 26.64%. Ammonium carbamate is believed to be bioactive to increase transdermal permeation through 2 mechanisms at once. The first mechanism increases the rate of drug penetration through the stratum corneum intercellular lipase and the second mechanism, decomposes rapidly to release two molecules-protonated dodecyl 6-aminohexanoate (DDEAC) and CO_2 [18]. These dual mechanism causes drug permeation through the lipid pathway, not through pores, thereby accelerating drugs distribution. Another benefit of this compound is notified as an active substance in the handling of specific bacteria. The combination of gallic acid and carbamic acid is effective in treating enteric bacterial [19]. It is not clear the function of ammonium carbamate compound in *M. hypoleuca*, related to strengthening self-defense. However, the closest explanation is defence against bacteria.

Levoglucosan compounds were found in the leaves part of 12.24%. Levoglucosan compound can be used as a parameter for the level of air pollution from biomass combustion above 300ºC [20] and affect human health [21]. The burning of biomass as heating source in winter season is a significant source of the presence of carbon aerosols in the atmosphere. The comparison of levoglucosan concentrations in winter in Belgium and Austria was 30 and 9 times, respectively, higher than those in summer [22]. Thus, levoglucosan is a typical compound from biomass combustion. The combustion mechanism in the Pyro-GCMS column is responsible for the present of this compound.

The organic compound of taraxerone (EH1) is well beneficial in traditional medicine. Taraxerone compounds are reported to have anti-microbial and anti-fungal biological activities [23,24]. Some researcher reported EH1 in *Euphorbia hirta* [23], *Discophora guianensis* [24] and *Hoya buotii* Kloppenb [25]. Carbinol compound is reported to have antimicrobial activity against pathogens *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Salmonella cholerae-suis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27583, *Escherichia coli* ATCC 25922 and *Shigella sonnei* ATCC 11060 and fungal pathogens *Candida albicans* ATCC 10231 [26] which commonly attacks humans. Carbinol was effective against *Pseudomonas palleroniana* R43631, *Bacillus* sp. R47065, R47131, *Paenibacillus* sp. B3a R49541, and *Bacillus simplex* M3-4 R49538 and elevate potato productivity [27]. In this discussion, taraxerone compounds become notable in strengthening *M. hypoleuca* defense system. However, the role of pentanoic acid compound in the Py-GCMS results cannot be clarified further. Pentanoic acid is an organic compound part of pheromones in mice as a warning function for carnivore prey [28].
Figure 2. Molecular structure in various parts of *M. hypoleuca*: (a) DBCP [29]; (b) 2-propenal methoxy [30]; (c) Ammonium carbamate [31]; (d) Levoglusan [32]; (e) Teraxerone [33]; (f) n-tetra teraxerone [34]; (g) carbinol [35]; (h) Pentanoic acid [36].

3.1. Terpenoid groups
Terpenoids group divided into hemiterpenoids (C5), monoterpenoids (C10), sesquiterpenoids (C15), diterpenoids (C20), sesterterpenoids (C25), triterpenoids (C30), tetraterpenoids (C40) and polyterpenoids (C> 40) [37–39]. The terpenoid compounds found in *M. hypoleuca*, based on carbon atom number, are listed in Table 2.

| Terpenoid compound | % relative |
|--------------------|------------|
| **Leaf**           |            |
| 2-metoksi-5-propeny, C_{10}H_{12}O_2 | 1.74 |
| Guanosin, C_{10}H_{13}N_5O_5 | 1.31 |
| Limonen, C_{10}H_{16} | 1.46 |
| Asam lakton, C_{10}H_{16}O_2 | 5.05 |
| trans-5,6-Epoksidekan, C_{10}H_{20}O | 0.32 |
| d-Nerolidol, C_{15}H_{26}O | 2.62 |
| n-Pentakosan, C_{25}H_{52} | 0.58 |
| 1-Siklo leikosan, C_{20}H_{52} | 0.45 |
| Terakseron, C_{30}H_{48}O | 6.75 |
| Squlen, C_{30}H_{50} | 0.92 |
| Alpha-Beta-D-Mannosid, C_{33}H_{60}O_7 | 0.77 |
| Pentatriakontan, C_{35}H_{72} | 0.24 |
| **Sap**            |            |
| Terakseron, C_{30}H_{48}O | 11.71 |
| Alpha-Beta-D-Mannosid, C_{33}H_{60}O_7 | 1.49 |
| **Stem**           |            |
| 2-propenal, C_{10}H_{10}O_3 | 35.69 |
| Isoeugenol, C_{10}H_{12}O_2 | 6.11 |
| 1-(4-Hidroksi-3-MET), C_{10}H_{12}O_3 | 2.06 |
| 4-Metil-2,5-Dimetoksisenzaldehyde, C_{10}H_{12}O_3 | 3.16 |
| Asam homovanillik, C_{10}H_{12}O_4 | 1.48 |
| Asetosringon, C_{10}H_{12}O_4 | 0.96 |
| Guaicol, C_{10}H_{14}O_2 | 0.97 |
This study identified several terpenoid compounds that are typical characteristic of secondary metabolites of higher plants class. Concentrations of 59% and 22% are derived from sap and leaves, with relative natural pesticide. DBCP was used extensively on agricultural crops, but was prohibited due to risks to human health. The constituents of DBCP were derived from sap and leaves, with relative concentrations of 59% and 22%, respectively. This study identified several terpenoid compounds that are typical characteristic of secondary metabolites of higher plants class.

4. Conclusion

*M hypoleuca* has complete defenses against pests and diseases, promote it an excellent pioneer plant. At least 100 organic compounds were identified from leaves, sap, wood, bark of *M. hypoleuca*. Dibromo chloropropane (DBCP) emerged as major compound in defense system against pests and diseases, as a natural pesticide. DBCP was once used intensively on agricultural crops, but was prohibited due to risks to human health. The constituents of DBCP were derived from sap and leaves, with relative concentrations of 59% and 22%, respectively. This study identified several terpenoid compounds that are typical characteristic of secondary metabolites of higher plants class.

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### Table: Terpenoid compound

| Terpenoid compound                      | % relative |
|----------------------------------------|------------|
| 3-(p-hidroksi-m-metoksifenil)-2-propanal, C_{10}H_{10}O_{3} | 19.49      |
| Fenol, 3-allyl-2-metoksi-, C_{10}H_{12}O_{2}                | 1.78       |
| Eugenol, C_{10}H_{12}O_{2}                | 2.12       |
| 2-Propanon, 1-(4-hidroksi), C_{10}H_{12}O_{3}                | 2.51       |
| Dimetoksibenzaldehid, C_{10}H_{12}O_{3}                | 2.48       |
| Guanosin, C_{10}H_{13}N_{5}O_{5}                | 2.07       |
| Dekanon, C_{10}H_{20}O                | 1.67       |
| Sikloheksileikosan, C_{26}H_{52}            | 0.64       |
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