Ethnopharmacology and chemical screening of piper from ketambe research station, Gunung Leuser National Park, Southeast Aceh

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Abstract. Throughout human history, people used various materials from nature to cure their illnesses and improve their health. Substances were derived from flora, fauna and mineral sources located in people’s immediate surroundings but also remote areas. Still, there is very little information on ethnopharmacology and publications about plants from Piper, especially those from around Ketambe. The research was done by exploration method, direct observation and interview, followed by laboratory analysis and literature study. Phytochemical screening was performed by the Ciuley Method. In this exploration, there were seven species of Piper. The results of interviews with indigenous elders obtained information that the seven Piper species can be used to cure different diseases. From phytochemical screening, it was found that the seven Piper species studied contained sterols & triterpenoids, carotenoids, tannins, cumarin derivatives, polyuranide, steroid glycosides but did not contain fat & high fatty acids, emodols/anthracids and coumarins.

Keywords: ethnopharmacology, ketambe, phytochemical, piper, screening

1. Introduction
The Ketambe Research Station (Ketambe RS) is located within the Gunung Leuser National Park Area (GLNP). Administratively, this station is in the area of Bale Lutu Village, Badar District, Southeast Aceh Regency. Based on the geographical position lies on the coordinates 03°40’39” North Latitude and 97°39’13” East Longitude. The research area in Ketambe RS is about 450 hectares and is equipped with a trail system to facilitate researchers. This research area is limited by the Ketambe River in the west and Alas River to the east. Besides, the research area is surrounded by several mountains, namely Mount Kemiri in the north, Mamas Mountain in the west and Mount Bendahara in the east, with variations in height in general between 300-1000 meters above sea level. The average annual rainfall is between 2650-4700 mm with humidity 91-96%. Ketambe RS is the most accessible station compared to other research stations within the Leuser ecosystem [1].

Throughout human history, people used various materials from nature to cure their illnesses and improve their health. Substances were derived from flora, fauna and mineral sources located in people’s immediate surroundings but also remote areas [2]. Medicinal plants have gotten a lot of attention recently because of its utilization in treating common illnesses such as cold, fever and other medicinal claims, nowadays many have been supported by strong scientific evidence. The study of medicinal plants begins with extraction procedures that play an important role in test results (e.g.
phytochemical results and content) and also to the consequent assays performed [3]. Phytochemical screening is an analytical method to determine the types of secondary metabolites found in plants because of their characteristics that can react specifically to certain reagents.

This research was conducted because the information and publications about the species of Piper found and used as traditional medicine around GLNP especially around Ketambe RS, have not been widely publicized.

2. Materials and methods

2.1. Place and time
Field research was conducted in July 2009 around the forest area of Ketambe RS - GLNP, Southeast Aceh, Nanggroe Aceh Darussalam. Laboratory analysis was conducted in September - November 2009 at the Botanical Division, Research Center for Biology – Indonesian Institute of Sciences (LIPI).

2.2. Materials and tools
The plant materials used in this study were leaves of seven Piper species found around Ketambe RS. The chemicals used were distilled water, methanol, ethanol, chloroform, ether, saturated solution SbCl₃, chloralhydrate, concentrated hydrochloric acid, anhydrous acetic acid, ammonium hydroxide, NaOH, KOH, concentrated sulfuric acid, FeCl₃, Stiasny reactants, Dragendorf reagents, Libermann-Bouchard reagents, and Mayer reagents.

The tools used were meshed sieve 100, funnel, test tube, volatile plate, pipette, stirrer, erlenmeyer, measuring cup, analytical balance, pH meter, centrifuge, UV lamp, water bath, Retsch Muhle’s grinding machine brand, Nikon's binocular microscope, and its equipment.

2.3. Procedure
Field research was conducted through direct exploration and observation in the forest. Information on these Piper species was obtained from experts and interviews with surrounding communities who know and utilize the plant traditionally, followed by a literature study. Collections were carried out, including collections for herbarium specimens that would later be used for plant identification as well as Piper leaf sampling for further analysis in the laboratory.

2.4. Plant determination
Specimens of plants to be investigated were identified in Herbarium Bogoriense, Research Center for Biology - LIPI, Bogor.

2.5. Preparation of simplicia
Leaves are cleansed of impurities attached, then cut into small pieces and dried. The drying process stopped when the leaves can be easily broken. There was an effort to maintain the water content in raw material about 5-10%. It was expected at that water content, most fungi can not grow. The dried material then powdered using a grinding machine, to easily assist the penetration of solvent onto the cellular structure of the plant, to help secondary metabolite dissolution and broaden extraction field [4]. Furthermore, the powder obtained was collected and stored in a sealed container and ready for further analysis.

2.6. Phytochemical screening
Phytochemical screening was performed by Cuiley Method [5]. The powder was extracted using diethylether then with ethanol, and finally with distilled water so that extracts of ether, alcohol, and water were obtained. The three extract types were qualitatively analyzed their chemical compounds.

2.6.1. Ether extract. 25 grams of powder was weight and then extracted with diethyl ether. The filtrate formed was filtered and collected. This procedure was repeated until clear colored extraction resulted.
The ether extract was concentrated to ± 50 ml for further chemical content identification. The residue/dregs were first dried for further extraction using an alcohol solvent.

2.6.2. Alcohol extract. The dried residue from extraction using ether was refluxed 2-3 times of 70-80% ethanol for 20-40 minutes. The result of filtration was collected and concentrated to ± 50 ml for further chemical content identification.

2.6.3. Water extract. Residue from extraction with alcohol was dried, accompanied by warm distilled water for 30 minutes, filtered and concentrated to ± 50 ml. The chemical content identification was then carried out. The same way was done on the juice of hydrolyzed water.

3. Results and discussion

Traditional medicines and cosmetics have been known and used by the common public since ancient times. Now there is an increasing trend in their use, but unfortunately, only a small portion has been scientifically researched. Therefore, to meet the needs of the community for medicinal and cosmetic ingredients, it is necessary to pay attention to traditional medicines and cosmetics derived from plants [6]. Research on traditional medicinal and cosmetics plants has recently been highly developed to obtain new cosmetics and drugs with pharmacological properties that are better than those currently available. Besides that, it helps the government program in conserving medicinal plants and traditional medicine. According to WHO records nearly 80% of the world's population is still dependent on plants to treat diseases and maintain their health, especially in developing countries [7].

*Piper* is one of the genera in the *Piperaceae* family which includes more than a thousand plant species that are spread in tropical and sub-tropical regions [7]. *Piper* is mostly herbaceous, sometimes woody plants that often climb using sticky roots. It has various phyllotaxis, simple, marginate, pinnate or palmate venation, often with hot and pungent odor. Flowers are arranged in inflorescent called *amentum*, small without perianth, unisex or bisex with 1-10 stamens, 1-6 pistils. The pistil has unicarpel upright at its base. The fruit is one-seeded berry with endosperm and perisperm. Seeds content essential oil cells [8].

Information from interviews with experts and the community around the Ketame RC, there can be found seven species of *Piper* that have the potential for medicine (figure 1 and table 1), namely merica hutan (*Piper* sp.1), akar cengkado (*Piper* sp.2), cengkado merah (*Piper* sp.3), pianang (*Piper* sp.4), belo uten (*Piper betle* L.), sirih merah (*Piper porphyrophylum* NE. Br.) and gume (*Piper umbellatum* L.). The part of the plant that is used as a medicinal ingredient in most of the *Piper* studied is the leaves, except in cengkado merah can be used all parts of the plant. The usefulness of each species of *Piper* plant is also diverse.

Ethnopharmacology was defined as “the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man” [10]. Medicinal plants are important elements of indigenous medical systems in many parts of the world, and these resources are usually regarded as a part of traditional knowledge of a culture [11].

The use of plants as ingredients for medicine, cosmetics, and aromatics is related to the chemical compounds in these plants. Chemical compounds contained in plants can be extracted optimally when extracted in solvents with an appropriate polarity level. Therefore, phytochemical screening needs to be done using solvents at various levels of polarity, so that it is expected that all chemical compounds in plants can be extracted properly. Phytochemical screening was carried out to determine the chemical content of the simplicia from seven species of *Piper* qualitatively (table 2).

If the plant contains active components called terpenoids or terpenes, it means that the plant has the potential to be used as an essential oil. The seven *Piper* studied contained essential oils except gume. But the seven *Piper* do not contain fat and high fatty acids. Fats are chemical compounds arranged by Carbon (C), Hydrogen (H), and Oxygen (O), insoluble in water so to dissolve fat requires special solvents such as ethers, chloroform, and benzene [14].
Figure 1. Seven species of Piper which have the potential as medicinal ingredients [9].

Table 1. The use of Piper as medicinal and cosmetic ingredients from Ketambe RS⁹

| No | Local Name       | Species               | Family        | Use                                                                 |
|----|------------------|-----------------------|---------------|----------------------------------------------------------------------|
| 1  | Merica hutan     | *Piper* sp.1          | *Piperaceae*  | Leaves: for relieving stroke symptoms (Interview)                     |
| 2  | Akar cengkado    | *Piper* sp.2          | *Piperaceae*  | Leaves: medicine for possessed people (Interview)                     |
| 3  | Cengkado merah   | *Piper* sp.3          | *Piperaceae*  | All parts of plants: cure a knife or glass wound (Interview)          |
| 4  | Pianang          | *Piper* sp.4          | *Piperaceae*  | Leaves: bladder disease (Interview)                                   |
| 5  | Belo uten        | *Piper betle* L.      | *Piperaceae*  | Leaves: water flea medicine (Interview); anti-bacterial, anti-fungal, prevent tumors [12] |
| 6  | Sirih merah      | *Piper porphyrophyllum* NE. Br. | *Piperaceae*  | Leaves: drug for diabetes and trance (Interview)                      |
| 7  | Gume             | *Piper umbellatum* L. | *Piperaceae*  | Leaves: eye medicine for children (Interview); fresh leaves: abscess, wounds, bruises, stomach ache, diuretics; leaf extract: eye conjunctivitis; fruit: chewed with betel for cough medicine; leaves and fruit: kidney disease, wasting water and stomach aches [13] |

Sumber: Kuncari ES, 2015

All *Piper* studied here are positive for sterols & triterpenoids. Sterol is a subgroup of steroids and an important form of biomolecules. They are present in all types of life forms, from plants, animals to mushrooms [15]. Triterpenoids are secondary metabolite compounds whose carbon skeleton comes from six isoprene units and is derived from acyclic \( C_{30} \) hydrocarbons. These compounds are cyclic or
acyclic and often have alcoholic groups, aldehydes, or carboxylic acids [16]. Most triterpenoid compounds have prominent physiological activities so that in everyday life they are widely used as medicines for the treatment of diabetes, menstrual disorders, snake contact, skin disorders, liver damage, and malaria. Plants that contain triterpenoid compounds are ecologically valuable because these compounds work as anti-fungus, insecticides, anti-predators, anti-bacterial and anti-virus [17].

| Identification                      | Merica Hutan | Akar Cengkado | Cengkado Merah | Pianang | Belo Uten | Sirih Merah | Gume |
|-------------------------------------|--------------|---------------|----------------|---------|-----------|-------------|------|
| Ether Extract                       |              |               |                |         |           |             |      |
| Essential oil                       | +            | +             | +              | +       | +         | +           | -    |
| Fat & High fatty acids              | -            | -             | -              | -       | -         | -           | -    |
| Sterols & Triterpenoids             | +            | +             | +              | +       | +         | +           | +    |
| Carotenoids                         | +            | +             | +              | +       | +         | +           | +    |
| Alkaloid alkaline                   | +            | -             | -              | -       | -         | -           | -    |
| Flavonoid aglycones                 | -            | -             | +              | -       | +         | -           | -    |
| Emodol/anthracenoid                 | -            | -             | -              | -       | -         | -           | -    |
| Coumarin                            | +            | +             | +              | +       | +         | +           | +    |
| Alcohol Extract                     |              |               |                |         |           |             |      |
| Tannin                              | +            | +             | +              | +       | +         | +           | +    |
| Reducing sugar                      | -            | -             | -              | -       | +         | +           | -    |
| Alkaloid salt                       | +            | -             | -              | +       | +         | -           | -    |
| Hydrolyzed alcohol extract          |              |               |                |         |           |             |      |
| Emodol/anthracenoid                 | -            | -             | -              | -       | -         | -           | +    |
| Coumarin derivatives                | +            | +             | +              | +       | +         | +           | +    |
| Steroid glycosides                  | +            | -             | +              | -       | +         | +           | -    |
| Flavonoids                          | -            | -             | +              | -       | -         | +           | +    |
| Water extract                       |              |               |                |         |           |             |      |
| Polyuranide                         | +            | +             | +              | +       | +         | +           | +    |
| Reducing sugar                      | +            | +             | +              | +       | +         | +           | -    |
| Saponin                             | -            | +             | -              | -       | -         | -           | -    |
| Tannin                              | +            | +             | +              | +       | +         | +           | +    |
| Alkaloid salt                       | +            | -             | -              | +       | +         | +           | +    |
| Extract of hydrolyzed water         |              |               |                |         |           |             |      |
| Coumarin                            | -            | -             | -              | -       | -         | -           | -    |
| Emodol/anthracenoid                 | -            | -             | -              | -       | +         | -           | -    |
| Flavone                             | -            | -             | +              | -       | -         | +           | -    |
| Steroid glycosides                  | +            | +             | +              | +       | +         | +           | +    |

- = not exist, + = exist

Carotenoids were detected positively in all *Piper*. Carotenoids are divided into carotene and xanthophyll. Carotene is the pigment that causes orange color, while xanthophyll causes a yellow color. Carotenoids can protect plants against solarization by absorbing excess light energy and then
released as heat. Carotenoids have very high antioxidant activity which will have an impact on the increased immune system [18].

The seven Piper studied were all positive for tannin. Tannins are phenol, have bitter taste and ability to tan leather. Tannins can react with proteins, some tannins are shown to have antioxidant activity, inhibit tumor growth and can inhibit the activity of enzymes such as reverse transcriptase and DNA topoisomerase. Other tannins can poison the liver [17]. Tannins can also be used as astringents, both for the digestive tract and skin. Besides, it is also an antidiarrheal drug. Physiological and pharmacological effects of tannins are caused by their ability to form complexes, both with proteins and polysaccharides [19].

Phenolic compounds and flavonoids function as antioxidants. According to Pokorny [20], antioxidants can neutralize free radicals by breaking the free radical chain reaction so that free radical reactions can be stopped. The search for natural ingredients that contain these compounds needs to be done to meet human needs for additional antioxidants. Robinson [17] defines that flavonoids include many of the most common pigments in the plant world. Some of its roles include growth regulators, photosynthetic regulators, antimicrobials and antiviral agents, which can also be abnormal components formed in response to infection/injury.

Polyuranides were detected positively in the seven Piper studied. Whereas other chemical content tends to vary between the five Piper studied. Alkaloids are usually found in plants as salts of various organic acids. Pharmacological actions of various alkaloids include analgesics, stimulants and some can increase blood pressure [21].

The results of qualitative phytochemical screening have not been able to prove the safety of the seven Piper studied if they will be used as raw materials for medicine, cosmetics, aromatic, food or feed. Although empirically, the community around Ketambe RC has proven its security, further testing is needed regarding the quality, efficacy and toxicity test.

**Conclusion**

The seven species of Piper studied empirically safe to cure different diseases. The results of phytochemical screening show that the seven species of Piper studied contained sterols & triterpenoids, carotenoids, tannins, coumarin derivatives, polyuranide, steroid glycosides but they did not contain fat & high fatty acids, emodols/anthracenosid and coumarins.

Further research is needed to obtain data on quality, efficacy, and toxicity so that medicinal plants can be called rational herbal medicines. Further testing can be done, among other things with benefit tests, preclinical tests or clinical trials.

**Acknowledgments**

Thank you to the head of the Research Center for Biology–LIPI for research collaboration with funds from the General Directorate of Higher Education - Ministry of Education; Drs. Razali Yusuf as the coordinator of the Ketambe research project; Head of the Leuser Ecosystem Area Management Agency and all staff and experts (especially Mr. Abdullah), as well as all local people who have assisted in the research and publication of this paper.

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