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Phytochemical and Histochemical Screening of Toxic Plant Based on Knowledge of Tengger Tribe in Ngadiwono Village, Pasuruan

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Abstract
Around hundreds of plant's species have been confirmed to produce toxic that could endanger the life of human or domestic animals. Some of those plants have potentials to be developed as medicine. The objective of this study is to reveal the secondary metabolite content of toxic plants mentioned by Tengger tribe on Ngadiwono village through phytochemical and histochemical screening test. This study was conducted on Ngadiwono village, Tosari district, Pasuruan Regency. Informants were selected using snowball method by following the information of key person (n informant=14). Plants were selected and collected according to the local knowledge, then was analyzed qualitatively for its secondary metabolite content through phytochemical and histochemical screening test. The results showed that there are 8 plant species categorized as toxic by local people, including Bedor (Girardinia palmata), Kecubung Bunga Kuning (Brugmansia suaveolens), Kecubung Bunga Putih (Brugmansia suaveolens), Jarak (Ricinus communis), Terpasan Kuning (Cestrum elegans), Terpasan Merah (Cestrum elegans), Kudisan (Euphorbia pulcerrima), Ciplukan (Physalis peruviana). The phytochemical result indicated that all toxic plants mentioned by the

local people contain alkaloid substancess, while histochemical test showed that alkaloid substances was found in leaf trichomes, except in Terpasan Merah (Cestrum elegans).

Keywords: Fitochemical, histochemical, Toxic plant,Tengger Tribe

INTRODUCTION(
Most of the Tengger tribe live by depending on the environment, including the utilization of biodiversity plants and animals [1]. The knowledge of Tengger tribe about land and resource management is mainly affected by history, custom, and available resources [2]. The biological resources utilized by Tengger tribe is including plants that have been utilized by human all over the world for a long time. Therefore, the interaction between human and plant is very important [3].
The knowledge of plants becomes very important recently along with the appearance of many kinds of diseases that threaten the human life. For example is the production of new medicines developed from coumpounds in plants. There are more than hundreds toxic plants, and some of them have potentials to be developed as medicine. Some species of toxic plants also utilized as pest control, such as nicotine in Nicotiana sp., seed extract of Baringtonia sp., and rotenoid in Derris spp. [4]. Chondodendron tumentosum contains of toxic alkaloid substancess called D-Tubocurarine. This toxic usually used by Indian society in Amazon to create poisoned arrows. Further, that substances is developed as muscle relaxant for surgery [5]. Surprisingly, all of those natural substancess become the basic for developing new medicine with a better quality.
Reflecting the potency of secondary metabolite in toxic plants as revealed by previous study, therefore the aim of this study is to identify secondary metabolite in toxic plants according to the information from Tengger tribe in Ngadiwono village.
MATERIAL AND METHOD
This study was performed on Ngadiwono village, Tosari district, Pasuruan regency, East Java, Indonesia (34'20'35.29

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N). Ngadiwono village is a buffer zone of Bromo Tengger Semeru National Park that comprised of 4 sub-districts: Ledoksari, Krajan, Ketuwon, and Banyu Meneng. Total area of the village is 639.03 ha. The distance between settlement area and forest is 2 km. The minimum temperature reached 10'C. Total number of male is 1097 individual, while female is 1474 individual, and the population density is 402 [6].
Data of toxic plant species was collected using semi-structured and in-depth interview. Fourteen informants were determined using snowball method. The researchers were accompanied by local people during data and sample collection. Data collection was terminated if it already got saturated data [7]. Stems, leaves, seeds, and flowers were collected for each species. Secondary metabolite test was conducted by following the

procedure described below.

Sample Preparation

Leaves were dried using oven in the temperature of 60' for 2 days. While seeds were dried using oven in the temperature of 60' for 3 days.

Alkaloid Test

Two grams of sample powder were extracted using little chloroform. Sample was then added with 10 ml of chloroform-ammonia and was filtered. The collected filtrate was added with drops of H2SO4 2M, homogenized until it formed 2 layers. Acid layer (colorless) was moved into three new glasses reaction. Each solvent was tested using drops of Dragendorf, Mayer, and Wagner reagents. The results were categorized as positive if the solvent forming precipitate with color of orange (Dragendorf), yellowish white (Mayer), and brown (Wagner) [8].

Flavonoid Test

Samples were soaked in N-Hexane and filtered. The residue was added with N-Hexane and filtered.

filtrate color turned into colorless. Then, filtrate was added with methanol, filtered, added concentrated HCl and Mg powder. If the filtrate form red brick precipitate, then the plant positively contains flavonoid [8]

Terpenoid and Steroid Test

Samples were soaked in N-Hexane and filtered. Then, filtrate was evaporated until forming residue. Filtrate was then added with chloroform 0.5 ml, acetic acid anhydrous 0.5 ml, and concentrated H2SO4 1-2 ml. If it forms reddish purple precipitate, then the plant positively contains terpenoid. In contrary, green precipitation means that the plant positively contains steroid [9].

Tannin and Saponin Test

Samples were soaked in N-Hexane, filtered, and added with N-Hexane until its color turned into green. Then, filtrate was added methanol, filtered, added ethanol, and filtered again. Ethanol filtrate was divided into two tubes.

FeCl3 3. If the filtrate turns its color into blackish green, then the plant positively contains tannin. The second tube was heated, then shaken. If it produces foam, then the plant is positive to contain saponin [10].

Histochemical Test of Transverse Leaf Sections

Leaf samples were cut into transverse section about 20-25 µm using microtom. The section was examined using reagents and then observed under Olympus BX51 microscope. Alkaloid content was examined using Bauchardat reagent. The positive result of alkaloid is indicated by the presence of reddish brown or yellow [11].

RESULT AND DISCUSSION

Phytochemical Analysis

The result of this study revealed 8 toxic Plants that usually utilizes by local people. They are bedor (Girardinia palmata), terpasan merah (Cestrum elegans), terpasan kuning (Cestrum elegans), jarak (Ricinus communis), kecubung putih (Brugmansia suaveolens), kecubung kuning (Brugmansia suaveolens), ciplukan (Physalis peruviana), Kembang kudis (Euphorbia pulcherrima). The toxic parts and the symptoms of poisoning of the plants mentioned above is presented in Table 1. The results of phytochemical screening on plants considered to be toxic by the Ngadiwono villagers are presented in Table 2.

Phytochemical tests performed are qualitative, so that the results only able to describe the group of substancess without more specific information about the type and concentration of secondary metabolite. Alkaloid test on jarak seeds (Ricinus communis) produces more alkaloid precipitate according to Gupta [12], risins, such as toxalbumin, phorbol, and cyanic acid. Kecubung (Brugmansia suaveolens) contains alkaloid scopolamine [13].

that all toxic plants mentioned by local people contained alkaloid substancess. Petersen [14] described several types of alkaloid substancess based on the structure of the molecular ring as well as 12000 alkaloid chemicals. Each alkaloid substancess will cause different symptoms of poisoning. The general symptoms of alkaloid poisoning were fever, anxiety, dilated pupils, reddened skin, dry skin, hallucinations and gastrointestinal symptoms. That symptoms could be occurred in both human and livestock. The screening results also found steroids and terpenoids in the toxic plants. Fahraud et al. [15] stated that all those substancess are toxic to stomach.

Histochemical Analysis of Toxic Plant

that several plants cause different poisoning symptoms. Bedor has thorn that causes itching if touched by the skin. Hidayat [16] revealed that Urtica Family, such as Bedor, have trichomes specialized into hair that could induce itchiness. Trichome are made up of long, broad-based cells that swell, narrowed, and pointed for its top (Figure 1). The tapered end wall contains silica, while the lower part contains calcium. If it is touched, the rounded part on the end of tapered part will break at the boundary are, and the pointed part will be easily cut through the skin and then the chemical substances (histamine and acetylcholine) will cause itchiness.

Histochemical identification of Brugmansia suaveolens leaves found alkaloid substances at the trichome of capitate gland. The trichome of capitate gland comprised of 1 to 2 head cells with varying stalks, from the shortest until the longest contained 1 to 3 cells. That results also found in various plant species, such as Lavandula pinnata L. [16]. Trichome cells at peltate and capitate glands in T. quinquecostatus secretes substances that is similar to alkaloids [17]. On the leaves of Physalis peruviana,

trichomes of uniseriat gland was found to be containing alkaloid. Alkaloid substances is found at the cross-sectional of Cestrum elegans leaves (yellow flower) in the secretory epidermis. In contrary, Cestrum elegans leaves (red flower) did not have it.
According to the result of phytochemical test, Cestrum elegans leaves contained alkaloid substances. In contrary, histochemical result did not detect any alkaloid compond in the plant's tissue. The presentage of metabolite concentration is predicted to be increasing along with the growth of the plants. However, adult organs have higher concentration compared to old organs that is experiencing degradation on its secretion structure [18]. Leaves samples used in this study did not collected based on the leaves age, so it affected the substances concentration in each leaves tissue. Besides, phytochemical test used the whole leaves, while histochemical test only observed the secretory structure of leaves tissue.
Basically, all plants experiencing secretion. Secretion is the event of separation of a number of substances from protoplasm or isolation inside several protoplasm. The secreted substances could be in from of excessive ion that is separated in a form of salt. Excessive assimilation could be issued as sugar or substances in the cell wall, such as lignin, suberin, and chitin. In addition, secretions also included substances that are the final product of metabolism or not the final product, but can not be used or only half of it that can be used physiologically (alkaloids, tannins terpen, harsa, and various crystals), or substances that physiologically functionate after secretion (enzyme, hormone). Secretion includes the release of material from the cell (either the surface of the cell or the space in the plants), or the accumulation of secretions in one part of the cell. Secretion in plants is usually produced in hair glands, tubes, and latisifer (sap cells, latex cells) [16].

CONCLUSION

Phytochemical screening test resulted that all toxic plants in this study contained alkaloid. While steroids found in bedor leaves, terpasan kuning leaves, terpasan merah leaves, jarak leaves, kecubung putih leaves and seeds, and ciplukan leaves. Terpenoid substances found in terpasan leaves, jarak seeds, kecubung putih leaves and seeds, kecubung kuning leaves and seeds, and kembang kudis leaves. Flavonoids only found in terpasan kuning and kecubung putih leaves. According to histochemical test, trichome of toxic plant contained alkaloid, except for terpasan merah leaves (Cestrum elegans).

ACKNOWLEDGEMENT

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Table 1. List of toxic plant and symptoms of poisoning

No. Common Name Scientific Name Family Toxic Parts Symptoms of Poisoning 1. Bedor Girardinia palmata Urticaceae Thorns at leaves and stems Burning sensation in the skin 2. Terapasan Merah
Cestrum elegans Solanaceae Stems, leaves, and flowers Abdominal bloating and death in livestock 3. Terpasan Kuning Cestrum elegans Solanaceae Stems, leaves, and flowers Abdominal bloating and death in livestock 4. Jarak Ricinus communis

Euphorbiaceae
Leaves and seeds Abdominal bloating in livestock. Seeds cause hallucinations in human. 5. Kecubung putih bunga tidak rangkap/ kecubung hitam Brugmansia Suaveolens Solanaceae Leaves, seeds Abdominal bloating in livestock. Seeds cause hallucinations in human. 6. Kecubung Kuning Brugmansia Suaveolens Solanaceae Leaves, seeds Abdominal bloating in livestock. Seeds cause hallucinations in human. 7. Ciplukan Physalis peruviana Solanaceae Leaves Abdominal bloating in livestock. 8. Kembang kudis Euphorbia pulcherrima Euphorbiaceae Stem sap Itching on exposed skin

Table 2. Phytochemical identification result of secondary metabolite substances in toxic plants at Ngadiwono village

Common Name Secondary Metabolite Substances Alkaloids Flavonoids Tannins Steroids Terpenoids Saponins Bedor + - - + - - Terpasan Merah Leaves + - - + + - Terpasan Kuning Leaves + + - + - - Jarak Leaves + - - + - - Jarak Seeds ++ - - - + - Kecubung Putih Leaves + + - + + - Kecubung Putih Seeds ++ - - + + - Kecubung Kuning Leaves + - - - + - Kecubung Kuning Seeds ++ - - - + - Ciplukan Leaves + - - + + - Kembang Kudis Leaves + - - - + - Key: presence (+); plentiful (++); and absence (-)

Figure 1. The result of histochemical screening on the Leaves trichome of Girardinia palmata; (1) Control; (2) Alkaloid test, Brugmansia suaveolens (white); (3) Control; (4) Alkaloid test, Brugmansia suaveolens (white); (5) Control; (6) Alkaloid test, Cestrum elegans (yellow); (7) Control; (8) Alkaloid test, Cestrum elegans (red); (9) Control; (10) Alkaloid test, Physalis peruviana. (11) Control; (12) Alkaloid test, Euphorbia pulcherrima; (13) Control; (1) Alkaloid test.

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