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A COMPREHENSIVE STUDY AND ANTIMICROBIAL EVALUATION OF *ALPINIA EREMOCHLAMYS* K. SCHUM. (ZINGIBERACEAE), AN ENDEMIC GINGER SPECIES OF SULAWESI, INDONESIA

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Abstract

Antimicrobial activity of *Alpinia eremochlamys* K. Schum (Zingiberaceae) which is an endemic ginger species of Sulawesi, Indonesia was studied from May 2019 to March 2020. The research site was located in the montane forest of Lore Lindu National Park (LLNP) near Sedoa village, Lore Utara district, Poso regency Central Sulawesi Indonesia. Identification of plant specimen, plant extraction and antimicrobial activities were carried out at the laboratory of Plant Biosystematics and Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences. Tadulako University. Antimicrobial activities were assayed by using agar well diffusion techniques. *A.eremochlamys* is now recorded from a new locality, in Sulawesi. The comprehensive notes of comprising synonym, descriptions, distribution, habitat and ecology, specimen examined and photographs are provided. Leaf and rhizome extract of *Alpinia eremochlamys* has an inhibition activity on the cell growth of three pathogenic microorganisms such as *Salmonella thypi, Staphylococcus aureus* and *Candida albican*.

Key words: *Alpinia eremochlamys*, Zingiberaceae, Sulawesi and Antimicrobial evaluation.

Introduction

The genus *Alpinia* (named after an Italian botanist; Prospero Alpino, 1533-1617) is the largest, most widespread, and taxonomically complex member of the family Zingiberaceae, with 230 species occurring throughout tropical and subtropical Asia (Kress *et al*., 2005). These species are distributed from Sri Lanka and the Western Ghats of India, to China, Japan, all of Southeast Asia, the Pacific including Fiji, Samoa, and the Caroline Islands, as well as Australia including Northern New South Wales (Larsen *et al*., 1998; Oyen & Dung, 1990). Furthermore, these species often predominate in the understory of forests but are also important ornamental and medicinal plants (Kress *et al*., 2005).

Southeast Asia is a region of high plant diversity, with about 50,000 flowering plant species (Von Rintelen *et al*., 2017; Middleton *et al*., 2019), including the genus Alpinia. Several studies have reported the genus, distribution and abundance within this region. Lamb *et al*., (2013) reported 19 species in the Borneo region (including four endemic), while Backer (1968) reported 4 species in Java island. According to Newman *et al*., (2004), there are 168 species of *Alpinia* in the Malesia region, including 56, 34, and 16 species endemic to Papua, the Philippines, and Sulawesi, respectively. Meanwhile, Ardi & Ardiyani (2015) reported two new *Alpinia* species belonging to subgenus *Cenolophon*, *Alpinia macrocrista*, and *Alpinia pusilla*, from Sulawesi, Indonesia. Also, in Vietnam, 16 *Alpinia* species, were disclosed to play a significant role in the lives of the indigenes, for instance, *Alpinia galanga*, *Alpinia oxyphylla*, and *Alpinia conchigera*, traditionally used in folk medicines to treat various diseases (Hanh *et al*., 2016).

Alpinia eremochlamis K. Schum (synonym: *Etlingera pectinata* Ridl.ex. Holtum), a tropical ginger herbs species, is distributed in Sulawesi island (Newman *et al*., 2004), however, only little is known about this distribution. A study by Turner & Cheek (1998) showed *Alpinia eremochlamys* was firstly collected from Tondano, Nothern Sulawesi, by Meyer in 1871, and from Kendari peninsula, Southeast Sulawesi by Beccari in 1874. The species was also collected from Tomohon by Sarasin in 1884, and from Gunung Ambang NR, North Sulawesi by Kinho In 2011. Currently, an extensive collection of *Alpinia eremochlamys* is deposited in the Royal Botanical Garden Edinburgh (RBGE, UK) (https://www.gbif.org/occurrence/map?taxon_key=5302286).

However, only a few reports provide scientific information on various aspects of *A. eremoclhamys*, including the previous studies (Ramadanil *et al*., 2019; Pitopang *et al*., 2019), where the Zingiberaceae species was reported to have been used in herbal medicine by residents of Lore Lindu National Park, Central Sulawesi, Indonesia. In addition, Zubair *et al*., (2021) reported this species was an endemic genetic source from Central Sulawesi, with the potential to be developed as an anti-HIV drug.

This study, therefore, aims to investigate the morphology, ecology, and distribution of *A. eremochlamys*, as well as the species' antimicrobial activities against *Salmonella typhae* and *Staphylococcus aureus* (bacteria), as well as *Candida albicans* (yeast).

Materials and Methods

Site description and plant specimen: This study was conducted from May 2019 to March 2020, in the humid,

tropical montane forest of Lore Lindu National Park (LLNP) near Sedoa village, Lore Utara district, Poso regency Central Sulawesi, Indonesia. The LLNP is a protected area of about 220,000 ha, with relatively undisturbed interior forest habitats, and margins characterized by the mozaic of a lightly disturbed forest, degraded forest, forest gardens as well as plantations (Gradstein *et al.*, 2007; Cicuzza *et al*., 2011; Pitopang, 2012). The park has several important functions, including habitat for Sulawesi's flora and fauna (Culmsee & Pitopang, 2009; Wanger *et al*., 2011), education and ecotourism destination (Manurung *et al*., 2019), as well as watershed protection (Leemhuis *et al*., 2007; Sutapa *et al*., 2018).

The population of *Alpinia eremochlamys* was studied using botanical exploration, while the morphological character and terminology were determined following the method by Stearn (1988) and Mabberley (2008). Furthermore, the vegetation was analyzed using the double plot method (Milliken, 1998), where the plot size was 10 X 10 m with five (5) replicates placed separately to observe tree species, with each plot containing nested plots of 5 X 5 m and 2m X 2m, established to record sapling species, as well as seedlings and understory plant species, respectively. Meanwhile, data on abiotic factors, including temperature, humidity, light intensity, and rainfall, were obtained from secondary sources (Fauzan *et al*., 2018).

All recognizable plant morphospecies were collected in sets of three (3) duplicates. Subsequently, the specimens were identified in the Laboratory of Plant Biosystematics, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, Indonesia, and the specimen vouchers specimens were stored in the CEB.

Extraction: Solvent extraction and phytochemical analyses of all the plant species parts were conducted using standard procedures (Harborne, 1973), at the Laboratory of Plant Biosystematics, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University. The plant vegetative (leaves, rhizome, and pseudostem) and reproductive (inflorescence and fruit of *A. eremochlamys*) organs were washed under running tap water, cut into 3 cm pieces, air-dried, and ground into powder, to expand the surface area, enabling optimal extraction. Subsequently, the leaf and rhizome powders were extracted by maceration with 95% ethanol, filtered, evaporated in a vacuum evaporator, then lypolized to obtain the yield of dry extract.

Microbial strain and culture media: For this experiment, two bacteria species, *Salmonella typhae, Staphylococcus aureus*, and one fungal species *Candida albicans*, selected based on clinical and pharmacological relevance (McCracken & Cowsan, 2003), were obtained from the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu. The bacteria stock cultures were subcultured in Nutrient Agar, while the *Candida albicans* cells were maintained on Potato Dextrose Agar (PDA) medium at room temperature.

Antimicrobial evaluation: The antimicrobial activity of the plant part extracts against two pathogenic bacteria *Salmonella typhae* (gram-negative) and *Staphylococcus aureus* (gram-positive) and one pathogenic yeast *Candida albicans* were investigated using the agar well diffusion technique (Alzoreky & Nakahara, 2003; Balouiri *et al*., 2016). The experiment used a Completely Randomized Design (CRD) with four different concentrations of the *Alpinia eremochlamys* extract (20, 40, 60, and 80%), as well as Chloramphenicol and grioseofulvin as positive standards (antibacterial and antifungal, respectively) and sterile aquadest as a negative control. Extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Subsequently, the diameter of bacteria and yeast inhibition zones around the disks were measured after incubation at 37°C for 18 to 24 hours.

Results and Discussion

Botanical description: Photographs and detailed notes of the examined specimen's synonym, descriptions, distribution, habitat, and ecology are provided below:

Synonym: *Alpinia pectinata* Ridl., J. Straits Branch Roy. Asiat. Soc. 34: 97 (1900), Turner and Cheek (1998), *Alpinia pectinata* Ridl. Gardens' Bulletin Singapore 50 (1998) 115-119.

Description: Perennial herb, of up to 5 m height, in loose clumps 10-15 cm apart, **Leafy shoots** 3-5 m long, with 2-14 leaves per shoot, base 10 cm in diameter, and dark red colour. Sheath colour orange, with scattered hairs, pubescence near margin, mainly glabrous margin, entire, black; ligule to 8-10 mm long, slightly entire, blackish, apex villose, sessile lamina, petiole 0 cm long, lamina green with yellow margins, densely villose on margin, lamina oblong crisped, 56-113 cm long x 6.5-15 cm, green, midrib pale yellow glabrous above and pubescence beneath, apex mucronate, base rounded, margin, and entire. Inflorescence 51-70 cm long, erect, secund, terminal, peduncle 50 cm long, dark black with sparsely orange, with up to 140 flowers and 25 fruits, Flower white, fertile bract 1.5 x 1.4 cm, pink in the center, glabrous. Flower 4 cm in long x 2 cm wide; calyx cup-shaped, 2 cm long, colour cream to yellowish, apex pale yellow, 1.4 cm long cream corolla tube, with sparse hair, corolla margin incurved, rounded, and cream; labellum elliptic, 1.5 cm x 0.9 cm, cream coloured in the middle, 2.3 cm long Stamen, 1.2 cm long filament, 10 x 6 mm anther, 0.5 mm wide stigma, colour yellow, glabrous; 1.3 mm long x 0.1 mm wide ovary, 0.1 cm long style, 0.5 cm wide stigma, yellow colour. Infructescence peduncle 8-12 cm, bracteole and calyx persistent, with 35-50 fruits per head, 0.7-2.3 cm long pedicel, bright golden, 2.0 x 1.6 cm fruit, globose, bright green, numerous seeds, pale white rounded. Rhizome very stout, 10 - 13 cm diameter, pubescent, young shoot up to 24 cm long, reddish-purple, with 3 cm ligule, purple-red, still roots absent (Fig. 1).

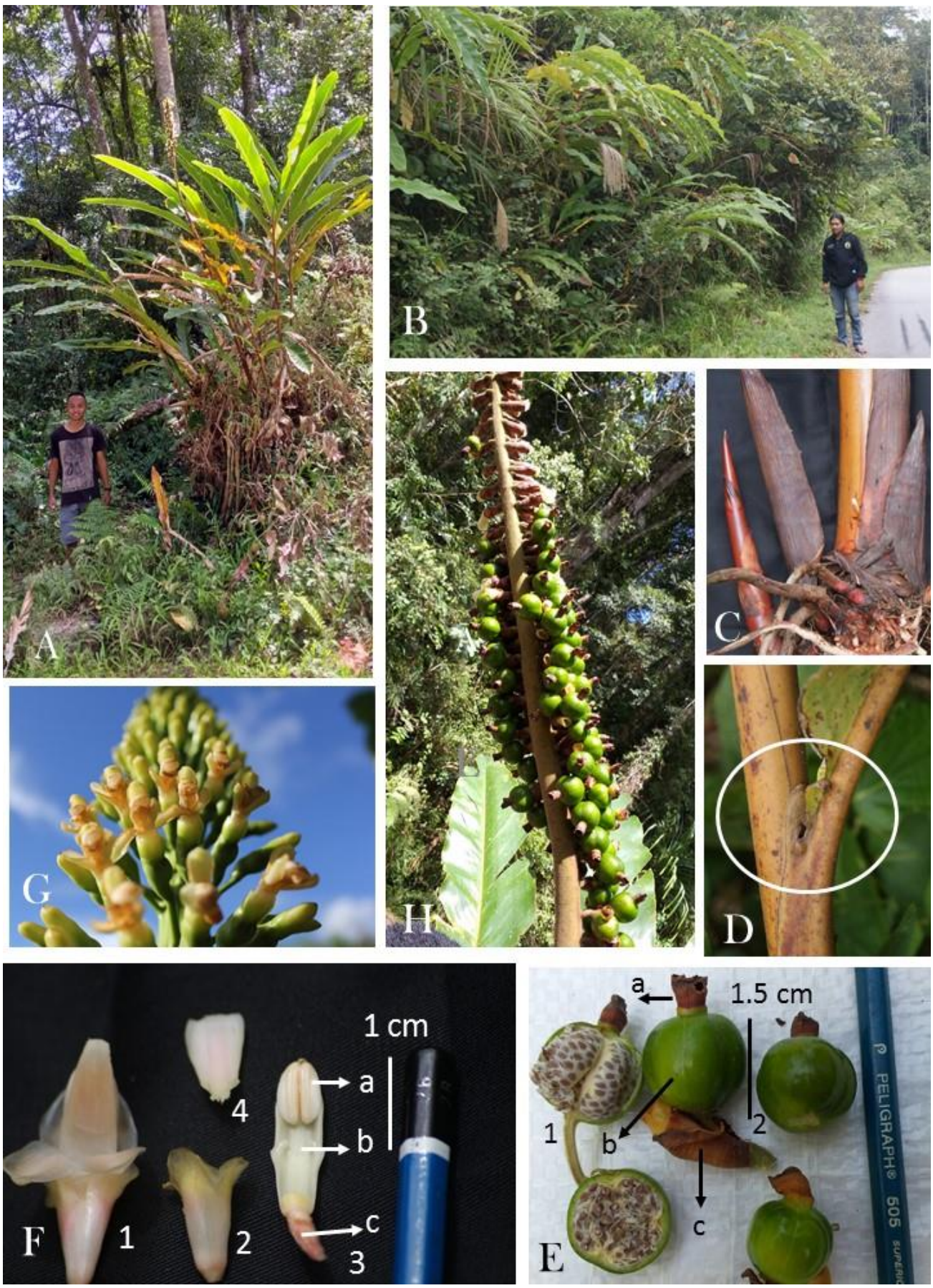

Fig. 1. A. *Alpinia eremochlamys* A.K. Schum. B. Habitat C. Leafy shoot with rhizome and young shoot D. Close up of ligule E. Fruit 1. Seed 2. Fruit. a.stigma, b. fruit c. Calyx F. Flower 1. Flower 2. Labellum 3. Antheridium (a. Anthera b. Fillamen), Gynaceum (c. Ovarium) 4. Calyx. All Photos from Ramadanil Pitopang (Ramadanil Pitopang *et al*., 10042 (CEB). G. Inflorescence H. Infructescence.

Fig. 2. Distribution map of *Alpinia eremochlamys* and the new locality in Sulawesi, Indonesia.

Distribution: Endemic to Sulawesi (Newman *et al*., 2004) Sulawesi distribution: Northern Sulawesi (Kinho, 2011), Southeastern Sulawesi (GBIF, 2020) and Central Sulawesi (Fig. 2).

Habitat and ecology: Based on the observation within the natural habitat where the specimen was collected, the species grows at the margin of the montane forest of Lore Lindu National Park, Central Sulawesi, at an altitude of 1200-1800 m.a.s.l. The area receives an annual rainfall of 1500-2000 mm and has an average minimum and maximum temperatures of 18-21°C, and 30-32°C, respectively, as well as an average relative humidity (RH) of 59.62 - 81.74% (Fauzan *et al*., 2018; Juhrbandt *et al*., 2010). Furthermore, the species is commonly found in the forest gaps or open areas, for instance, along the roadside between Dongi-dongi and Sedoa villages, Lore Utara Poso regency. Meanwhile, the habitat type was typically montane forest (Whitten *et al*., 2002), and several plant species were identified. Tree species were dominated by *Castanopsis accuminatisima* and followed by *Mallotus paniculatus* (Euphorbiaceae), while the poles species were dominated by *Elaeocarpus sphaericus* (Elaeocaraceae), *Pandanus sarasinorum* (Pandanaceae), as well as *Ficus virens* (Moraceae), and sapling species were dominated by *Vaccinium* sp. (Ericaceae), as well as *Melastoma malabathricum* (Melastomataceae). Numerous seedling and understorey plant species were also observed, including *Ageratum conyzoides* (Astercaeeae) *Begonia aptera* (Begoniaceae), *Coix lacrima-jobi* (Poaceae), *Spathoglotis plicata* (Orchidaceae), *Biden pilosa* (Asteraceae), *Tridax procumbens* (Asteraceae), *Clerodendrum* sp. (Lamiaceae) and *Rubus fraxinifolius* (Rosaceae).

Vernacular name: *Kasimpo* (Topo Baria languange, Sedoa, Lore Utara, Poso, Ramadanil Pitopang *et al.*, 10042 (CEB), and *Katimba* (Pamona languange, Poso).

Uses: *Alpinia eremochlamys* has been utilized traditionally by the *Topo Baria* tribe, an indigenous ethnic group in Sedoa village, located near the study area. The tribe refers to the plant as "Kasimpo", and uses the leaves as food wrapping while the decoction of the rhizome is used as a tonic (Pitopang *et al*., 2019; Ramadanil *et al*., 2019).

Phytochemical content: Based on the previous study (Ramadanil *et al*., 2019; Pitopang *et al*., 2019), the species' pseudostem contains several secondary metabolite compounds, including tannin, saponins, and terpenoids, while the rhizome contains flavonoids, tannins as well as saponins, and the leaves contain flavonoids, tannins, saponins, terpenoids as well as alkaloids.

The study area: Central Sulawesi, Poso regency, Lore Utara district, Sedoa village, Lore Lindu National Park, Danau Kalimpa'a, 01˚19.503'S, 120˚18.510'E, 1648 m elevation, on 5 October 2019. *Ramadanil Pitopang, Zulfadly & Adrianus Tombi* 10042 (CEB).

Antimicrobial evaluation: Based on the antimicrobial evaluation, *Alpinia eremochlamys* leaf and rhizome extract have a promising inhibition activity on the cell growth of *Salmonella typhae*, *Staphylococcus aureus*, and *Candida albicans*. Table 1 shows a statistical summary of the inhibition zones obtained in the three experiments.

According to Table 1, the inhibition diameter zone was formed the leaf and rhizome extracts of *A. flexuosa* on growth media containing the microorganism cultures. The rhizome extract of *A. eremochlamys* was discovered to have the highest inhibition on the growth of *C. albicans*, and the 40% rhizome extract produced inhibition diameters of 17.6 mm, 11.23mm, and 8.5mm, respectively, for C. *albicans*, *S. typhae* and *S. aureus*, respectively.

Table 1. Statistical summary of the Inhibition zone of *Alpinia eremochlamys* extracts against (A) *Salmonella typhae*, (B). *Staphylococcus aureus* and (C). *Candida albicans*. Different lower case letters in the same column indicate significantly different values (DMRT test).

No.	Treatment concentration (%)	Zone of inhibition (mm)		
		A	B	C
		Mean ± SD	Mean ± SD	Mean ± SD
1.	(Negative control)	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
2.	20	9.30 ± 0.32 b	3.3 ± 0.8 b	0.7 ± 7.2 a
3.	40	11.23 ± 0.25 c	8.5 ± 0.0 c	17.6± 0.4 b
4.	60	12.40 ± 0.36 d	12.7 ± 1.3 d	18.7 ± 1.8 b
5.	80	15.41 ± 0.51 e	17.7 ± 0.6 e	19.5± 2.0 b
6.	(Positive control)	30.70 ± 0.56 f	25.0 ± 0.5 f	28.9 ± 2.5 c

Notes: A represents the crude ethanolic extract of *A. eremochlamys* leaves against *Salmonella typhae*, using chloramphenicol as the positive control
B denotes the crude ethanolic extract of *A. eremochlamys* rhizomes against *Staphylococcus aureus* bacteria, using chloramphenicol as the positive control
C signifies the crude ethanolic extract of *A. eremochlamys* rhizome against *Candida albicans* yeast, using griseofulvine as the positive control

Discussions

Alpinia eremochlamys is a large clumped ginger species with terminal inflorescence and grows up to a height of up to 5m. The species has oblong-crisped, green leaves with yellow margins, while the inflorescence consists of numerous flowers with up to 140 flowers and 25 fruits of globose shape and green color. This species is endemic to Sulawesi (Newman *et al*., 2004), but has only been previously reported in north and southeastern Sulawesi. However, this study discovered the plant was also distributed in Central Sulawesi, precisely in the mountain forests of Lore Lindu National Park. This discovery indicates the botany research carried out in Sulawesi is inadequate.

Kessler *et al*., (2005) described Sulawesi (formerly known as Celebes) as the largest island the island with the least plant biodiversity density in the biogeographic Wallacea region, a unique world region identified as the hotspot of biodiversity (Keßler *et al*., 2002; Middleton *et al*., 2019; Sodhi *et al*., 2005). However, several novel plant species have been discovered on the island of Sulawesi within the past five years (Henderson *et al*., 2018; Ardi *et al*., 2019; Dayanti *et al*., 2020). The fastest-growing taxon is Begonia, with numerous species are identified each year (Ardi *et al*., 2019; Dayanti *et al*., 2020). Another significant taxon is Zingiberaceae, another group of plants being actively investigated (Poulsen, 2012; Ardiyani *et al*., 2017; Trimanto & Hapsari, 2018; Ardiyani & Poulsen, 2019), including the utilization (Sabilu *et al*., 2017; Pitopang *et al*., 2019; Ramadanil *et al*., 2019).

According to the field investigations, *Alpinia eremochlamys* is commonly found within the open area or forest gaps, along the margin of montane forest, Lore Lindu National Park, Central Sulawesi, at an altitude of 1200 to 1800 m a.s.l. Culmsee and Pitopang (2009) characterized this forest as a Fagaceae forest, and numerous plant species have been discovered in this area, including *Etlingera flexuosa*, *E. sublimata* (Poulsen, 2013), *Nepenthes pitopangii* (Lee *et al*., 2009), 5 new species of *Syzigium* (Brambach *et al*., 2017), and numerous tree species in Sulawesi (Culmsee & Pitopang, 2009). In addition, the forest area also serves as the habitat of various plants and animals species. Alpinias play an important ecological role in the understory of tropical and subtropical forests, where many species are commonly found in the understory, along forest margins, and in light gaps (Kress *et al*., 2005).

Based on the ethnobotanical studies, *A. eremochlamys* is used by natives of the Topo Baria community, for various purposes. The leaves are used as roofing material and food wrapping, while the rhizome is used to produce traditional medicine (Ramadanil *et al*., 2019). Numerous species from the genus *Alpinia* have been used by the community for various purposes, including medicine (Chouni & Pau, 2018), therapeutics (Victorio 2011), essential oil production (Kanjilai *et al*., 2010; Santos *et al*., 2012) as landscape ornamentals (Pitopang *et al*., 2019), spices and edible plants (Rachkeeree *et al*., 2018), customary ritual (Hariyadi & Ticktin, 2012), and flowers (Perez-Flores *et al*., 2017).

The antimicrobial assay showed *A. eremochlamys* leaf and rhizome extracts exhibited moderate antibacterial activity against *Salmonella typhae* and *Staphylococcus aureus*, respectively. Furthermore, the rhizome extract was also able to inhibit the growth of *Candida albicans*. Table 1 and Figure 4 show the antimicrobial activity of *A. eremochlamys*, seen from the formation of inhibitory zones.

Staphylococcus aureus is a ubiquitous bacteria species commonly found on the skin and hair as well as in the noses and throats of people and animals (Giaouris *et al*., 2015). These bacteria produce heat-stable enterotoxins responsible for 13-40% of the food poisoning cases within the United States, Canada, and Japan (Iniques-Mareno *et al*., 2018). Meanwhile, *Salmonella typhae* is a bacterial species responsible for typhoid fever (Mogasale *et al*., 2016) and *Candida albicans* is a fungus existing as a harmless commensal within the gastrointestinal and genitourinary tracts of healthy individuals (Nobile & Johnson, 2015).

Therefore, *A.eremochlamys* has the potential to be developed into botanical bactericides and fungicides. The impressive antimicrobial activity exhibited by *A.eremochlamys* against the three microorganism species is due to the plant's flavonoid, tannin, saponin, terpenoid, and alkaloid contents (Ramadanil *et al*.,

2019). Flavonoids are a large and structurally diverse group of naturally occurring compounds, and some of these compounds are present in propolis as well as honey used in some traditional medicine systems for the treatment of infectious diseases (Farhadi *et al.*, 2018). Meanwhile, tannic acids are a group of antioxidants used to treat bacterial diseases by destroying the cell wall's integrity, possibly to target the cell wall's peptidoglycan (Dong *et al.*, 2018).

In addition, saponins exhibit antimicrobial activity by inhibiting the growth of gram-positive and gram-negative microorganisms. These compounds also exhibit antifungal activity associated with the number and structure of monosaccharide units present in the sugar chains (Desei *et al*., 2009).

Conclusion

A.eremochlamys is a ginger species endemic to Sulawesi and was discovered in this study to be distributed in LLNP. This species is commonly found in the forest gaps and open areas, for instance, along the margin of the humid, tropical, montane forest of Lore Lindu National Park, Central Sulawesi, at an altitude of 1200-1800 m a.s.l. This species has been utilized by the *Topo Baria* tribe, for various purposes including food wrapping (leaves) and medicine (decoction of the rhizome). Furthermore, *Alpinia eremochlamys* leaf and rhizome extract have a promising inhibition activity on the cell growth of *Salmonella thyphae*, *Staphylococcus aureus* and *Candida albicans*.

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