

Full Length Research Article

Antimicrobial Activity of Ten Extractives from Toba, North Sumatra and Mt. Merapi National Park Regions, Indonesia

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ABSTRACT

Investigating beneficial chemical compounds of plant extracts is one of the ways to prevent biodiversity loss. This study evaluated the antimicrobial activity of indigenous plant extracts from Toba, North Sumatra, and Mt. Merapi National Park regions against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Candida albicans*, and *Candida tropicalis* by calculating the zone of microbial growth inhibition. Among the plant extracts, T5 that identified as *Toona sinensis* showed the highest microbial inhibition to the growth of *C. albicans*, *B. subtilis*, *S. typhi*, and *E. coli* with the diameter growth of approximately 2.00, 1.80, 1.33, and 1.33 cm, respectively. Based on those results, *T. sinensis* was then subsequently fractionated using n-hexane, ethyl acetate, and methanol, respectively. The resulted fractions also were evaluated for antimicrobial bioassay. All fractions have shown activity in inhibiting the growth of the microbes at 1% concentration. However, each fraction showed growth inhibition against certain microbes. The n-hexane fraction showed the greatest inhibitory activity for *E. coli* and *S. typhi*; ethyl acetate fraction for *B. subtilis* and *C. albicans*; and methanol fraction for *E. coli* and *S. typhi*. The results revealed that *T. sinensis* plant extract has great potential as an antimicrobial agent. Further investigation is needed to observe the mode of antimicrobial action of fractionated crude extracts of *T. Sinensis*. The exploring potency of Indonesian biodiversity opened up a new way for the utilization of plants for economic development and conservation.

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1. Introduction

Forest is vital for life because it serves as the habitat of the vast diversity of organisms. The biodiversity of forests is also economically important for humans, mostly for wood products such as timber, pulp, paper, and rubber. In addition, forest plant extracts have hidden medicinal effects that have not been well explored. However, in recent years, biodiversity loss is threatening forest areas because of climate change and global warming. Global warming affects evapotranspiration in the dry area leads to severe drought (Trenberth et al. 2014). Forest is prone to fire when drought is occurred, causing damage to seedlings, sprouts, young and old trees. The recovery and rehabilitation process will be difficult, resulting in biodiversity loss (Nasi et al. 2002).

Exploring forest plants and investigating the benefit of chemical compounds of plant extracts is one of the ways to prevent biodiversity loss. Plant extracts are reported to have medicinal effects such as antioxidant (Attanayake et al. 2016), antibacterial (Ogbole et al. 2018), antifungal (Giordani et al. 2020), and anticancer (Sammar et al. 2019). It also has been used as therapeutic drugs for degenerative diseases such as diabetes (Deo et al. 2016) and cardiovascular (Qadir et al. 2018). Advanced research in the pharmaceutical industry shifts people's perspectives on medicinal plants from unregulated traditional usage to phytopharmaceutical which is qualitatively and quantitatively standardized (Bhatt 2016). This leads to increasing demands for plant-based medicine.

Knowledge of the benefit of ethnobotanical plants (as medicinal, antimicrobial, and bioinsecticide) is expected to increase people's awareness to conserve the forests. People will begin to appreciate forests by using them as medicinal plant sources and will implement them as local culture. Local culture will encourage the creation of a legal system to prevent anything that damages the forests, such as wildfire or human activities. Therefore, it will help in avoiding biodiversity loss.

In line with the Indonesia Biodiversity Strategy and Action Plan (IBSAP) program, the exploration of plant extract that has the potential as bio-insecticides based on the local wisdom of the community have been conducted in Toba-North Sumatra (Meisyara et al. 2019) and Turgu Forest Area, Mt. Merapi National Park, Java. In this study, the collected plants in the previous project were screened to discover another potential activity as an antimicrobial agent.

2. Materials and Methods

In line with the Indonesia Biodiversity Strategy and Action Plan (IBSAP) program, the exploration of plant extract that has the potential as bio-insecticides based on the local wisdom of the community have been conducted in Toba-North Sumatra (Meisyara et al. 2019) and Turgu Forest Area, Mt. Merapi National Park, Java (Ismayati et al. 2019) (Fig. 1). About 4 plant samples were collected from Mt. Merapi, and 6 plant samples were collected from the Toba regions (Samosir Botanical Garden and Eden 100 Park Area). All samples were extracted by maceration process to get methanol crude extract, and followed by a phytochemical test (Meisyara et al. 2019).

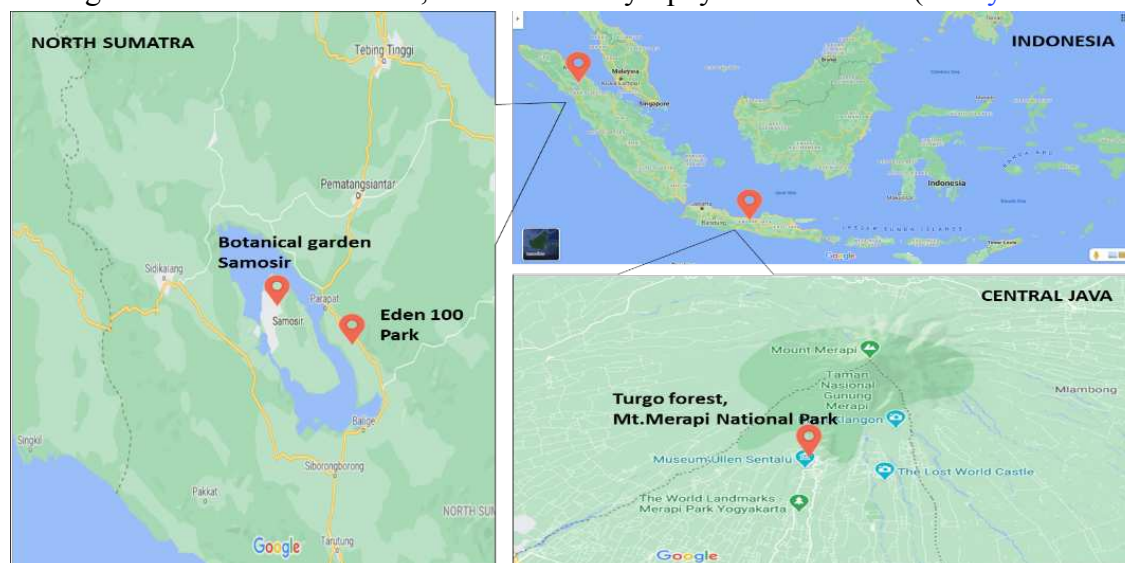


Fig. 1. Sampling location in Toba, North Sumatra and Mt. Merapi National Park, Java, Indonesia (Source: www.maps.google.com)

Antimicrobial bioassay for 10 plant extracts was carried out against 6 microbial pathogens, including *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* (bacteria), and *Candida albicans*, *Candida tropicalis* (yeast) using the disc diffusion method. Microbial pathogenic agar plates were made by mixing 1% of each bacterial and yeast inoculum with a density of about 10^7 cfu/ml, respectively, with 20 ml molten agar media (nutrient agar for bacteria and potato dextrose agar for yeast) and then pour it into petri dishes. 50 μ l plant extract (10,000 ppm in methanol) was injected onto a paper disc (0.8 cm) and placed on the microbial inoculated agar. Methanol was used as a control. The plates were then incubated at 25°C for 48 h. Observation of microbial inhibition zone diameter (clear zone formation) was carried out at 24 and 48 h.

The plant extract with the highest antimicrobial activity was fractioned by the gradient polarity of the solvent. Ethyl acetate was chosen as the semi-polar solvent, and *n*-hexane was used for nonpolar solvent. Each extract with concentration of 0.1%; 0.2%; 0.4%; 0.6%; 0.8% and 1.0% w/v were subjected into the same antimicrobial test following the aforementioned methods.

The effects of plant extractives treatment on the inhibition zone of pathogen microbial were statistically determined by analysis of variance (ANOVA), and samples showing significant differences were analyzed using Duncan's post hoc test. All analyses were performed using IBM SPSS version 2.2.

3. Results and Discussion

Biodiversity plays a significant role in the development of the country. In line with the objectives of the 2015–2020 IBSAP program, the exploration of biodiversity in the archipelago has a role in increasing people's productivity and national competitiveness, and economic independence. The results of this exploration are expected to be used as material for analysis in the management and utilization of sustainable biodiversity to achieve development targets.

From this exploration activity, 6 plant extracts from the Toba area and 4 plant extracts from the Merapi area were collected. The plant extracts were identified and reported in previous studies (Ismayati et al. 2019; Meisyara et al. 2018). They are leaves and barks of kina (*Cinchona pubescens* Vahl), leaf of kamadoh (*Dendrocnide stimulans* (L.f.) Chew), and keremi (*Homalanthus populneus* (Geiseler) Pax); leaf of simar pahit-pahit (*Tithonia diversifolia* (Hemsl.) A. Gray), imar huting-huting (*Chromolaena odorata* (L.) R.M. King & H. Rob), pirdot (*Saurauia bracteosa* DC.), Ingul (*Toona sinensis*), haurese (*Azadirachta indica* A. Juss), and bark of sikkam (*Turpinia sphaerocarpa* Hassk).

The chemical content analysis of plant extracts has been carried out for the phytochemical test (**Table 1**). Each plant extract showed various content of phytochemicals, although none of the extracts contain triterpenoid. Variation in pathogen growth inhibition may be influenced by differences in the chemical content of the plant extract samples.

The results of the antimicrobial activity of plant extracts are shown in **Fig. 2**. The ANOVA indicated that all plant extracts showed antimicrobial activity against bacterial pathogens tested with various potencies. *T. sinensis* (T5) extract at 10,000 ppm was found to be the most effective in suppressing all bacterial and yeast pathogenic growth tested in vitro. In contrast, extract of keremi (*Homalanthus populneus* (Geiseler) Pax) exhibited antibacterial activity only against *S. aureus*. Other plant extracts were variously effective against bacterial pathogen *E. coli*, *B. subtilis*, and *S. aureus* but showed no inhibitory effect on the growth of *S. typhi* and yeast pathogens. The significant differences ($p = 0.001$) in the mean of growth inhibition zone for those three pathogens

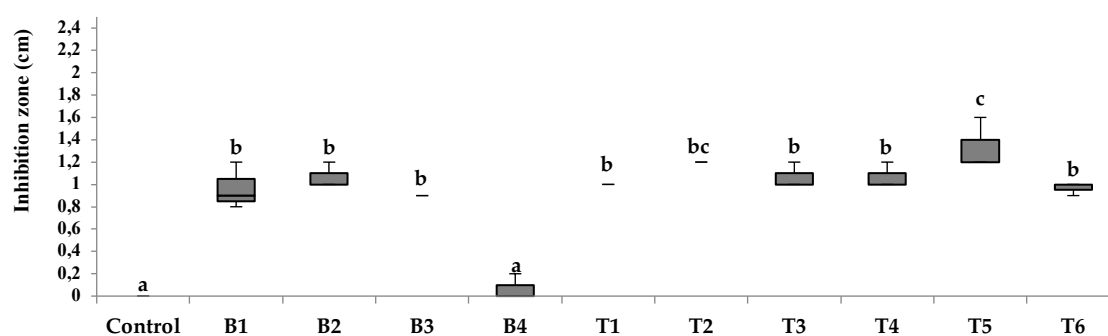
were only observed at the treatment of T5. The clear zone of *S. typhi*, *C. albicans*, and *C. tropicalis* after treated with 10,000 ppm T5 were 1.90 cm; 1.20 cm; and 0.40 cm, respectively.

Table 1. The phytochemical test result of plant extractives collected

Code	Location	Local name Yield (%)	Phytochemical test result					
			Alkaloid	Flavonoid	Steroid	Triterpenoid	Saponin	Tannin
B1	Mt. Merapi National Park, Java (Ismayati et al. 2019)	Kina (<i>Cinchona pubescens</i> Vahl)	+	-	-	-	+	-
B2		Kina (<i>Cinchona pubescens</i> Vahl)	+	+	-	-	+	+
B3		Kamadoh (<i>Dendrocnide stimulans</i> (L.f.) Chew)	-	-	-	-	+	-
B4		Keremi (<i>Homalanthus populneus</i> (Geiseler) Pax)	-	-	-	-	-	+
T1	Toba region, Sumatera (Meisyara et al. 2019)	Simar pahit-pahit (<i>Tithonia diversifolia</i> (Hemsl.) A. Gray)	-	-	+	-	-	-
T2		Simar huting-huting (<i>Chromolaena odorata</i> (L.) R.M. King & H. Rob)	-	+	-	-	-	+
T3		Pirdot (<i>Saurauia bracteosa</i> DC.)	-	-	+	-	-	+
T4		Sikkam (<i>Turpinia sphaerocarpa</i> Hassk)	-	+	-	-	-	+
T5		Ingul (<i>Toona sinensis</i>)	-	-	+	-	-	+
T6		Hauresse (<i>Azadirachta indica</i> A. Juss)	-	+	+	-	+	+

T. sinensis showed the highest inhibitory against all pathogens tested above and the result is in accordance with the activity of *T. sinensis* as antitumor, hypoglycemic, antioxidant, anti-inflammatory, antibacterial, and antiviral, as reported by Peng et al. (2019). According to phytochemical test, *T. sinensis* has steroid and tannin that might act as active compound against the pathogen. Similar results showed that *T. sinensis* activity against *S. aureus* was due to the presence of sesquiterpenes (84.64%), including copaene (8.27%), β -caryophyllene (10.16%), caryophyllene (13.18%), and β -eudesmene (5.06%) (Wu et al. 2014). The difference in terpenoid compounds that did not appear in the chemical analysis may be correlated to the different location growth and age of the plant (Ismayati et al. 2017).

Escherichia coli



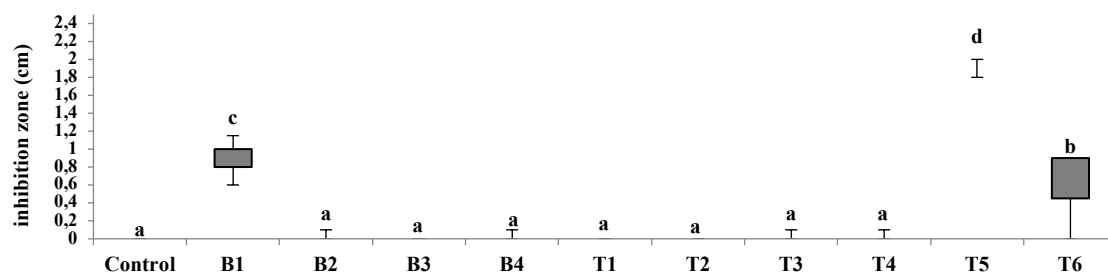
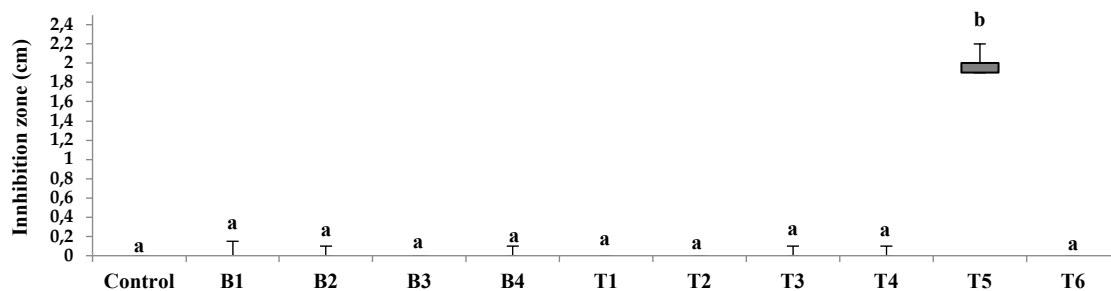
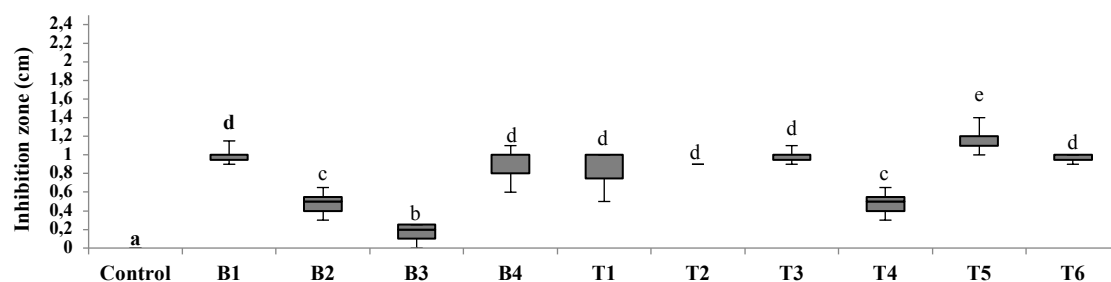
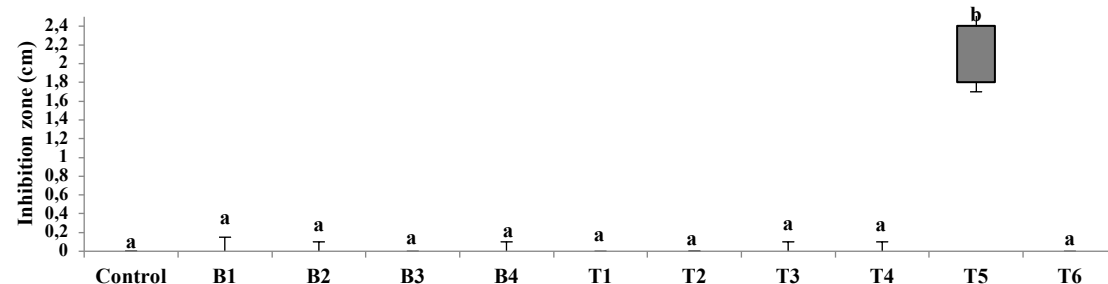
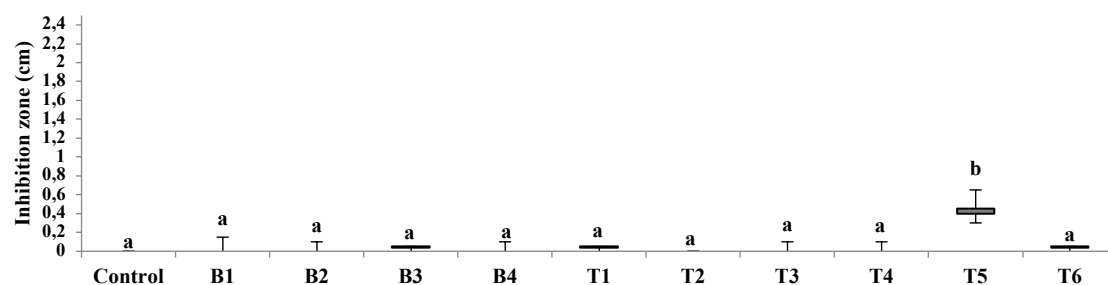
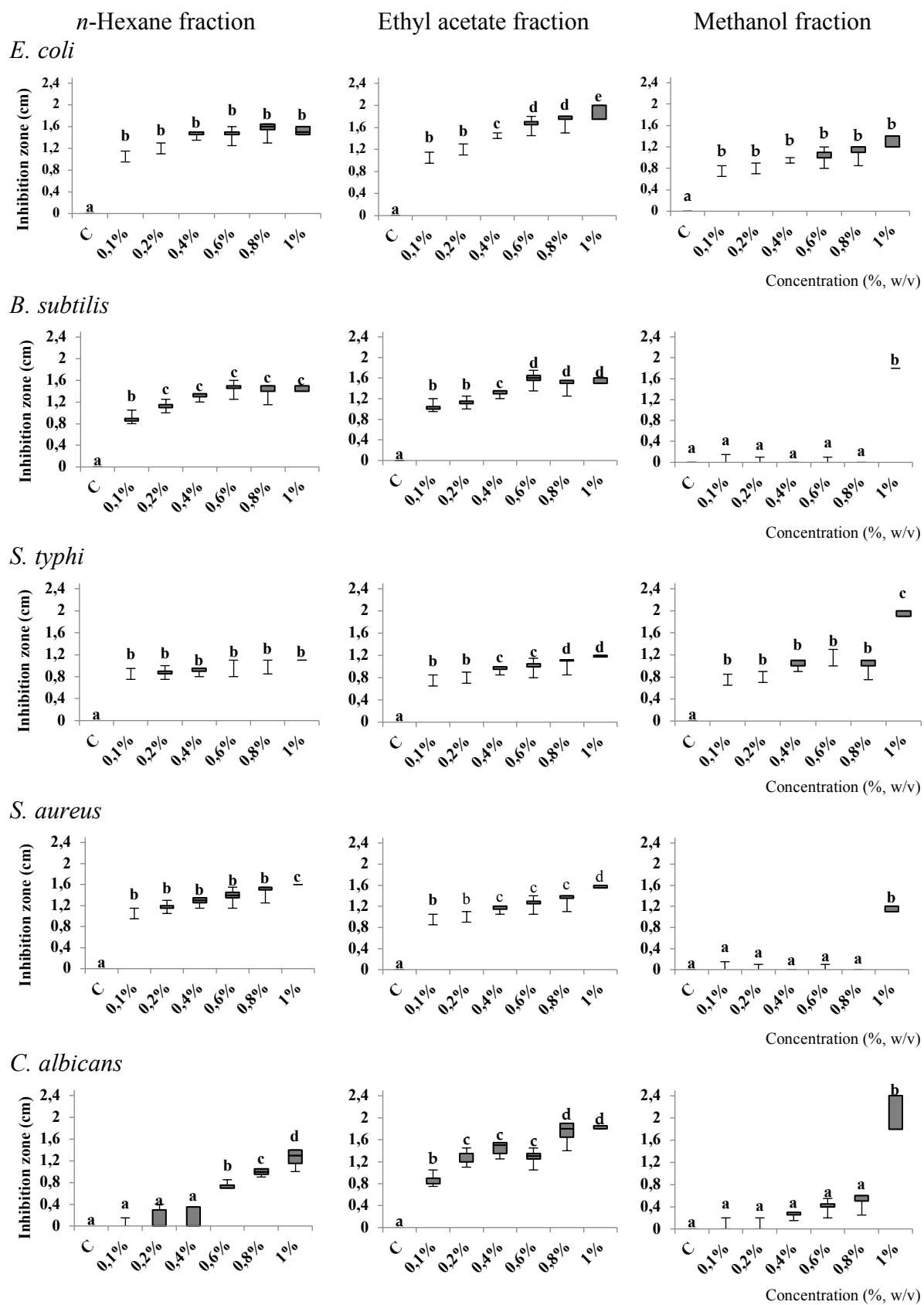
Bacillus subtilis*Salmonella typhi**Staphylococcus aureus**Candida albicans**Candida tropicalis*

Fig. 2. Box plot of inhibition zone of bacteria and yeast in antimicrobial bioassay in some crude extractives, 1% (w/v) (Legends: Treatments with the same letters in the same graphic are not statistically different at $p < 0.05$, as determined by Duncan's test; sample codes are referred to **Table 1**).

Although in this study, other extracts did not achieve antimicrobial activity as good as *T. sinensis*, previous studies reported that some of the extracts also have considerable antimicrobial activity. *Tithonia diversifolia* showed great antimicrobial activity on a particular microbial pathogen as *Pseudomonas aeruginosa* (Oso and Ogunnusi 2017). On the other hand, *Azadirachta indica* extract showed antimicrobial activity against Gram-positive bacteria and favorable for foodborne pathogens and spoilage organisms (Mahfuzul Hoque et al. 2007).

The antimicrobial activity of plants was believed originally from their secondary metabolites. The secondary metabolites of nine collected plants were analyzed with a phytochemical test and the result is showed in **Table 1**. Plant-alkaloids, such as sanguarine, tomatidine, reserpine, piperine, and cinchona alkaloids, are reported to have high antimicrobial activity against a broad range of bacteria (Barbieri et al. 2017). Flavonoids have promising antimicrobial activity, such as selectively targeting bacterial cells, restricting virulence factors, and biofilm formation (Górniak et al. 2019). Steroids from two *Chromolaena* species (Taleb Contini et al. 2003) and *Nerium oleander* (Archit et al. 2014) showed activity against Gram-positive bacteria. Triterpenoids isolated from *Dissotis senegambiensis* and *Amphiblemma monticola* showed antibacterial activity against methicillin-resistant *S. Aureus* (Nzogong et al. 2018). Saponin extracts from fifteen indigenous plants from Turkey are reported effective against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Staphylococcus aureus*, *Candida albicans*, *Candida parapsilosis*, *Candida pseudotropicalis*, and *Candida stellatoidea*. Tannins' mode of action against bacteria includes inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial growth, or direct action on microbial metabolism through inhibition of oxidative phosphorylation, also through iron deprivation (Scalbert 1991).

The results suggested that *T. sinensis* extract showed a strong antimicrobial effect against pathogens strains. Therefore, to further clarify the effect of extractive content of *T. sinensis*, the crude extract was fractionated into polar and nonpolar fractions using gradient solvent in polarity (*n*-hexane, ethyl acetate, and methanol). Obtained fractions were then exposed against all pathogens. As seen in **Fig. 3**, the growth inhibition zone's diameter tends to increase by higher fraction concentration. On *n*-hexane fraction, the zone's diameter in all pathogen seems to similar for all concentrations. A significant difference was seen at a concentration of 0.6% (w/v) and above, with the inhibition zone's diameter about 0.8–1.6 cm. Meanwhile, the diameter zone shown a slightly higher than *n*-hexane, it is about 2.0 cm. A significant difference effect was also found in phenolate content in *T. sinensis*, polar extract (*n*-butanol and ethyl acetate, 36.42 and 31.64 mg) have a higher content than nonpolar solvent (*n*-hexane, 10.06 mg) (Murningsih 2015). A strange phenomenon has occurred in the bacteria *S. aureus* and *B. subtilis*, where only at a concentration of 1% the addition of the extract can inhibit the growth zone. As gram-negative bacteria, both of them are protected from certain physical assaults and do not absorb foreign materials that surround them as easier as gram-positive bacteria (Segun et al. 2015). Meanwhile, the ethyl acetate fraction showed high inhibition with clear zone diameters of about 1.65 cm and 1.55 cm in *C. albicans* and *C. tropicalis*.



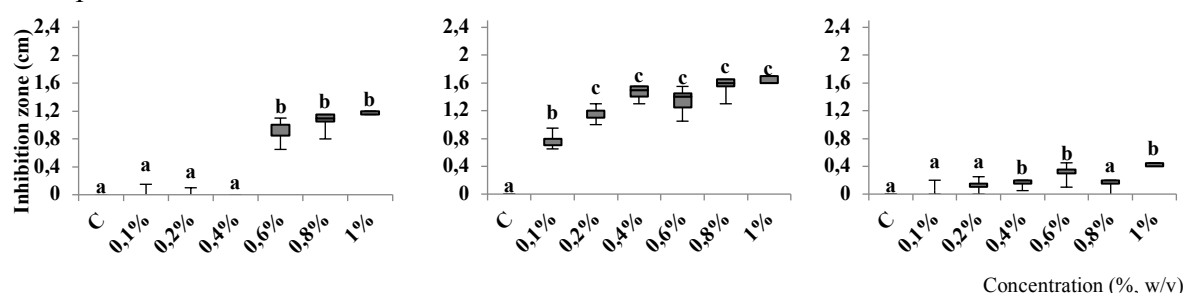
C. tropicalis

Fig. 3. Box plot of inhibition zone of bacteria and yeast in antimicrobial bioassay for *n*-hexane, ethyl acetate, and methanol fractions (Legends: Treatments with the same letters in the same graphic are not statistically different at $p < 0.05$, as determined by Duncan's test; sample codes are referred to **Table 1**).

Further study is needed to explore the potency of antimicrobial activity related to chemical composition. The production of standardized extracts from plants is a real potential for improving local communities' economy and important information in terms of plant conservation.

4. Conclusions

The present study showed that all samples collected from Toba, North Sumatra, and Mt. Merapi national park have potential antimicrobial. *Toona sinensis* (T5) was presented as the most active in inhibiting the growth of *C. albicans*, *B. subtilis*, *S. typhi*, and *E. coli*, with the diameter growth about 2.00, 1.80, 1.33, and 1.33 cm, respectively. The *n*-hexane fraction shows the greatest inhibitory activity for *E. coli* and *S. aureus*; ethyl acetate fraction for *E. coli*, *B. subtilis*, and *C. albicans*; and methanol fraction for *E. coli* and *S. typhi*. Further investigation is needed to observe the mode of action onto microbial growth that caused by the chemical content of *T. sinensis*.

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