Biological activity of local plant extracts from Toba Region as insecticide

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Abstract. Toba, North Sumatera is famous not only for its tourism destination but also for biodiversity. The aim of this research is to explore the potency of the local plant from Toba as bioinsecticide. Six local plant samples, which consists of genus Compositae, Meliaceae, Staphyleaceae, and Actinidiaceae, were obtained from the Toba region. The macerated extracts were tested their insecticidal activity against Coptotermes gestroi and Spodoptera litura. Bioassay results showed that leaves extract of Ingul (Toona sinensis) at a concentration of 10% (w/v) had the highest mortality effect against Coptotermes gestroi on the tenth day of observation. Whereas, leaves extracts of Pirdot (Saurauia bracteosa), Ingul (Toona sinensis), and Haurese (Azadirachta indica) at the concentration of 1% (w/v) showed the best insecticidal effect with 80% mortality percentage respectively against Spodoptera litura in 4 hours of observation.

1. Introduction
Public awareness of a healthy lifestyle is increasing, and it leads to a growing demand for organic foods. Willer et al. [1] reported that the global organic food market was increasing by 500% from 2000 to 2016. Hence, organic farming needs support to maintain its production. The industry of organic foods needs pesticide from natural sources or biopesticide. Plant has potency as biopesticide since it produces secondary metabolite compounds. Plant metabolite compounds are known to be effective as a control against some important insect pests such as termite [2], cotton leafworm [3], mosquito [4], and tick [5]. Therefore, these compounds can be used as insect pest management. Their mode of action against insect pests is through disrupting their neuron-system, such as cholinergic system, GABA (gamma-aminobutyric acid) system, mitochondrial system, and octopaminergic system, also their hormonal balance [6]. Moreover, the advantages of using botanical compounds as an insecticide in place of synthetic insecticide are that they are environmentally friendly, target-specific, suitable in integrated pest management programs, and sustainable [7].

There are four botanical products mostly used for insecticide, which are pyrethrum, rotenone, neem, and essential oils [8]. These products are globally being produced as they have resulted in good effects for insect control. However, the utilization of these products continuously for a long period induces resistance in insect pests. Therefore, the discovery of new botanical sources as an insecticide is needed to replace the current insecticidal compounds.

Toba in North Sumatera is a region in Indonesia which is famous for its tourism destination and biodiversity, especially local plants. For centuries, they have been used by local communities for daily needs. Ethnobotany study on this area reported that there are 163 species of local plants usually used by
subethnic Batak Toba in Peadungdung Village for medicine (92 species), food (71 species), firewood (25 species), symbol (23 species), local technology (18 species), livestock fodder (18 species), rope (15 species), art (11 species), and revenue (11 species) [9]. Research on Samosir Island has also found 48 plant species which have potencies as timber, ornamental, fruits, and traditional medicine [10]. Although many potencies of local plants have been identified, but their potency as a botanical insecticide is lesser-known. Hence, the aim of this research is to expand knowledge or information of local plants in Toba Region which are potential to be developed as a bioinsecticide.

2. Materials and methods

2.1. Sample collection and identification
Exploration of biological resources that have the potential as bio-insecticides based on the local wisdom of the community in the Toba-North Sumatra regions was carried out using a questionnaire method to the local community assisted by information from the local government. Herbal plants that are thought to have the potential for insecticide were prepared as much as 5 kg for each type of plant and sent to Research Center for Biology, LIPI for identification.

2.2. Sample preparation
Plant leaves and barks were shade dried at ambient temperature of 32 °C ± 5 °C till materials moisture content reached ± 12%. The materials were pulverized using a disc mill and sieved (40–80 mesh size). The sieved materials were extracted in three stages; each stage extraction used agitated maceration method (72 h, 150 rpm, 30 °C) with methanol (Merck, Darmstadt, Germany) as a solvent in 1:7 ratio. The extracted solvent was concentrated by evaporation to obtain crude extractive. The phytochemical test was conducted for secondary metabolites, alkaloids, flavonoids, steroid, triterpenoid, saponin, and tannin.

2.3. No-choice feeding tests against Coptotermes gestroi
No-choice feeding test was conducted according to Ohmura et al. [11]. A test container was made of a plastic petri dish (diameter 6cm) filled up with 3 mm plaster of Paris at the bottom and moistened with deionized water. Paper discs (diameter 13 mm; Whatmann International) were treated with each plant extract with 10% concentrations. The control discs were untreated. The discs were dried for 12 hours in a vacuum desiccator. Fifty workers and five soldiers, and treated paper disc were entered into a test container. The bioassay was carried out for 14 days. Termite mortality was observed every two days and in the final period observation, the mass loss of paper disc was evaluated.

2.4 Leaf-dipping bioassay method against Spodoptera litura
Bioassay against Spodoptera litura larvae was conducted according to Krishanti et al. [12]. Taro leaves were washed in running water and air dried. Subsequently, taro leaves were cut in square size (4x4 cm²), dipped in each extract solution for about one minute. After they were oven-dried for about 1 hour, the leaves were placed on filter paper layered in clean petri dish size (6x6 cm²). Ten third instar stage of S. litura was then put on the treated leaves in a petri dish. The trial was designed with three replications. Larval mortality post-treatment was recorded interval in 1, 2, 3, 4, 24 and 48 hours.

3. Results and discussion
Exploration of plants that have potency as bioinsecticides based on local wisdom was carried out using the method of interviewing the local community. Six plant samples were collected from 3 areas in the Toba region. Simar huting-huting, pirdot, and sikkam were collected from Eden Park. Ingul was collected from Dolok Jangga Village. Haurese and simar pahit were collected from Samosir Botanical Garden. The identification results of the samples are shown in table 1.
Table 1. Plant samples identification result.

| Sample code | Local name       | Plant part | Species name                  |
|-------------|------------------|------------|--------------------------------|
| Toba 1      | Simar pahit-pahit| Leaf       | *Tithonia diversifolia* (Hemsl.) A.Gray |
| Toba 2      | Simar huting-huting| Leaf     | *Chromolaena odorata* (L.) R.M King & H.Rob. |
| Toba 3      | Pirdot           | Leaf       | *Saurauia bracteosa* DC.       |
| Toba 4      | Sikkam           | Bark       | *Turpinia sphaerocarpa* Hassk.  |
| Toba 5      | Ingul            | Leaf       | *Toona sinensis*               |
| Toba 6      | Haurese          | Leaf       | *Azadirachta indica* A. Juss.  |

The extraction result has shown that each plant sample has a different yield percentage (table 2). The used of methanol for extraction probably affected the differences of yield percentage as it was attracted hydrophilic compounds from plant samples. Therefore, *haurese* with the highest yield percentage probably has more hydrophilic compounds than other samples.

Table 2. Yield percentage of plant extracts.

| Sample code | Local name       | Yield (%) |
|-------------|------------------|-----------|
| Toba 1      | Simar pahit - pahit | 6.7       |
| Toba 2      | Simar huting - huting | 7.5       |
| Toba 3      | Pirdot           | 17.2      |
| Toba 4      | Sikkam           | 27.0      |
| Toba 5      | Ingul            | 5.3       |
| Toba 6      | Haurese          | 41.5      |

It is also relevant with phytochemical test result (table 3) that *haurese* has the most abundant phytochemical compounds than other plant samples. The crude extract of *haurese* positively resulted in flavonoid, steroid, saponin, and tannin. This result is correlated with Dash et al. [13] that phytochemical result of methanol extract of *Azadirachta indica* or neem are reported to have alkaloid, saponin, tannin, glycoside, flavonoid, and reducing sugars as constituents. Reported active compounds of *Azadirachta indica* or neem are azadirachtin, nimbin, nimbin, nimbidin, nimbidol, sodium niminate, gedunin, salannin, and quercetin [14]. These active compounds are believed behind the many potencies of neem, i.e. anticancer, anti-inflammatory, antibacterial, antifungal, antioxidant, and as an insecticide [14]. According to Himmi et al [15], defatted neem oil formulation (DNO) achieved 7.5% of efficacy level in wood protection and soil barrier-test against termites.

Sikkam and pirdot extracts also resulted in a high yield percentage following haurese. Sikkam or *Turpinia sphaerocarpa* Hass is reported positive in alkaloid [16] and pirdot or *Saurauia bracteosa* DC. is reported positive in phenolic, flavonoid, and tannin [17].

Table 3. Phytochemical test results.

| Sample code | Local name       | Alkaloid | Flavonoid | Steroid | Triterpenoid | Saponin | Tannin |
|-------------|------------------|----------|-----------|---------|--------------|---------|--------|
| Toba 1      | Simar pahit- pahit| -        | -         | +       | -            | -       | -      |
| Toba 2      | Simar huting- huting | -      | +         | -       | -            | -       | +      |
| Toba 3      | Pirdot           | -        | -         | +       | -            | -       | +      |
| Toba 4      | Sikkam           | -        | +         | -       | -            | -       | +      |
| Toba 5      | Ingul            | -        | -         | +       | -            | -       | +      |
| Toba 6      | Haurese          | -        | +         | -       | +            | +       | +      |

Note: (+) positive; (-) negative
Figure 1 shows that six plant extracts have insecticide potency against *C. gestroi* compared to control (MeOH) and no-treatment groups. From the graphic below (figure 1), ingul or *Toona sinensis* has the best mortality effect on *C. gestroi* by inducing 100% mortality in just ten days of treatment. Following by ingul is simar pahit – pahit, haurese, pirdot, simar huting – huting, and sikkam sequentially.

![Termite Mortality (%)](image)

**Figure 1.** Termite mortality percentage of no-choice feeding test bioassay on *C. gestroi*.

On the other hand, mass loss percentage of paper discs (table 4) shows that compared to control, extract treatment decreased the appetite of termites. It shows that all extracts have antifeedant potency against *C. gestroi*. Simar huting – huting and ingul were completely avoided by termites with 0% mass loss percentage. Thus, those extracts have the highest antifeedant effect against *C. gestroi*. Whereas, pirdot has the lowest antifeedant effect among others with 8% mass loss percentage of paper disc.

| Sample code | Local name            | Mass loss percentage (%) |
|-------------|-----------------------|--------------------------|
| Control     | Methanol              | 22                       |
| Toba 1      | Simar pahit - pahit   | 2                        |
| Toba 2      | Simar huting - huting | 0                        |
| Toba 3      | Pirdot                | 8                        |
| Toba 4      | Sikkam                | 6                        |
| Toba 5      | Ingul                 | 0                        |
| Toba 6      | Haurese               | 3                        |

Leaf extracts of ingul or *Toona sinensis* are reported to have antifeedant activity on insect orders, i.e. Saltatoria, Heteroptera, Homoptera, Coleoptera, Lepidoptera and Diptera [18]. It also has a 90% mortality effect on adult *Drosophila melanogaster* [19]. Ingul in the phytochemical test (table 3) shows a positive result in steroid and tannin. These chemicals might involve in insecticide effect of *Toona sinensis* extract.

Observation of termite’s behavior during the treatment showed that the soldier secreted white oily-like substances onto paper disc containing plant extracts. Thus, these substances worked as a signal for workers to avoid the paper disc. These substances are believed as a defense mechanism of the soldier to protect the colony. Soldier caste in termite has a specialized task to defend the colony from the attack of other animals, or bacteria and fungi infection, and even from insecticide [20].
Morphology of soldier’s head and mandible allow them to counter the attack of other animals. Also, they can secrete chemical substances from their salivary and labral gland to defend the colony from bacteria and fungi infection, and insecticide. This chemical defense, depending on species, mostly consists of terpenoid, acetate-derived, and a few protein substances. Therefore, after observing termite behavior towards all plant samples, all the extracts probably have repellent potency against *C. gestroi* [20].

![Cotton Leafworm Mortality Percentage (%) within the initial 4 hours of observations](image1)

**Figure 2.** Mortality percentage of cotton leafworm within the initial 4 hours of observation.

![Cotton Leafworm Mortality Percentage (%)](image2)

**Figure 3.** Mortality percentage of cotton leafworm within two days of observation.

The bioassay result of *Spodoptera litura* (figure 2) showed that all extracts had a mortality effect on the insect. The increasing mortality percentage was observed at 4 hours of observation. The highest potency was shown by haurese, ingul, and pirdot extracts with ±80% mortality in 4 hours of observation. On the other hand, the lowest mortality percentage was shown by simar huting-huting with 63.3%. Observation of mass loss percentage of taro leaves showed that compared to control, each plant extract had different mechanism against *S. litura*. Pirdot with the highest mass loss percentage (even higher
than controls) showed that the extract did not lessen the appetite of *S. litura*. While figure 2 shows that pirdot had high mortality percentage in just 4 hours of observation. Therefore, pirdot probably has a toxicant effect against *S. litura*. On the other hand, haurese and ingul with high mortality percentage showed low mass loss percentage compared to controls. Hence, the two extracts probably have antifeedant potency against *S. litura*.

| Table 5. Mass loss percentage of taro leaves after extracts treatment. |
|-----------------------------|-----------------|-----------------|
| Sample code | Local name             | Mass loss percentage of taro leaves (%) |
|-------------|------------------------|----------------------------------------|
| Toba 1      | Simar pahit- pahit      | 67                                      |
| Toba 2      | Simar huting- huting    | 61                                      |
| Toba 3      | Pirdot                 | 70                                      |
| Toba 4      | Sikkam                 | 52                                      |
| Toba 5      | Ingul                  | 54                                      |
| Toba 6      | Haurese                | 63                                      |
| Control     | Methanol               | 64                                      |

Plant extracts have long been known to have a wide range of insecticide effects, such as antifeedant, repellent, and toxic effects. All of our plant samples showed an antifeedant effect on bioassay test against *C. gestroi* and *S. litura*. Antifeedant is any chemicals or substances that can deter feeding behavior of insects through their taste organs [21]. Triterpenoid is one of many insect antifeedants found in the plant extract. Limonoid from *Azadirachta indica* or known as azadirachtin or limonin from *Citrus* species is a few examples of well-studied triterpenoid as antifeedant against insects. Moreover, sesquiterpene, diterpene, triterpene, alkaloid, and phenolic are another secondary plant metabolites that have antifeedant activity against the insect. These natural substances from the plant can be used in pest management, replacing toxic and environmentally-dangerous insecticide [21].

4. Conclusion
Six local plants collected from Toba region and Hauresse (*Azadirachta indica*) resulted in the highest amount of extraction yield which was 41.5%. All the samples extract showed that the best three extracts for insecticide effect on *C. gestroi* and *S. litura* were Ingul (*Toona sinensis*), Pirdot (*Saurauia bracteosa*), and Hauresse (*Azadirachta indica*).

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