Phytochemical content, toxicity and antioxidant activities of native medicinal plants from North Sumatra

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Abstract. Sumatra Island, one of the major islands in Indonesia, has a high biodiversity of medicinal plants. The objective of the study was to identify and analyze the phytochemical content, toxicity, and antioxidant activity of ten medicinal plant species from North Sumatra. To find out the group of compounds, the plants were screened for the presence of phytochemicals i.e. tannins, saponins, flavonoids, triterpenoids, alkaloids, and hydroquinones. Antioxidant activity was measured by 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH). Results showed that the local people used the medicinal plant for various diseases or disorders such as stomach ache, fever medicine, fractures medicine, stomach ulcers, diuretic, and antidiabetic. The phytochemical testing showed that the extract contains flavonoids, saponins, tanin, and alkaloids. All medicinal plants are classified as toxic and have the potential as raw materials for medicines. The antioxidant activity (IC50) were Sikkam 27.06 ppm; Modang Kulim 104.81 ppm; Handis 35.38 ppm; Rugi-rugi 45.70 ppm; Sambang-sambang 207.63 ppm; Ampapaga 58.97 ppm; Sae-sae putih 53.19 ppm; Sae-sae hitam 41.08 ppm; Pirdot 146.40 ppm and Sirungguk more than 250 ppm. In general, the antioxidant activity of ten medicinal plant species studied has good activity. These plant species have the potential to be developed into standardized herbal medicines.

1. Introduction
Sumatra is one of the major islands in Indonesia with high biodiversity. It has 12 national parks from the north of the island of Sumatra to the southern. Conservation and buffer zones are attractive locations for researchers to study forest medicinal plants.

Forest medicinal plants are essential to be explored continuously because around 80% of the world community still depends on alternative medicines/herbal medicines. It is estimated that there are around 70,000 types of plants used for natural medicine [1].

Ethnobotany is the study of the dynamic relationship between plants and people [2]. Combining high throughput technology with a selection of plants based on ethnobotanical information is likely to succeed in finding new biological activities and compounds. It should consider ethnobotany a pharmaceutical research tool for the present and future with important perspectives in drug discovery [3].

Research on medicinal plants through ethnobotany search is a reasonably effective step in the framework of discovering new drug sources. Local people’s wisdom, which has been empirically practiced from generation to generation, can provide beneficial information. Collaborative work between academic ethnobotanists and indigenous specialists can benefit both communities and researchers. Ethnobotanical studies do not need to be confined to far-away places or different cultures; people everywhere have knowledge of plants [4].
One type of disease that affects many people is cancer. To prevent its disease, it makes efforts by consuming special diet such as antioxidant sources. Medicinal plants from forests contain many kinds of antioxidants. For example, the extract of *Cotylelobium melanoxylon* Pierre has antioxidant activity against DPPH radicals with the value of IC$_{50}$ as much as 108.487 ppm and 77.909 ppm for *Cotylelobium lanceolatum* Craib [5]. Research on several Indonesian medicinal plants showed that of the samples investigated, 29 exhibited radical scavenging properties more than 50% at 1000 ppm. Five extracts exhibited high activities i.e., bark extracts of *Sapium baccatum* and *Leucosyke capitellata*, leaves extracts of *Ardisia crispa*, *Glochidion cauliflorum*, and *Glochidion superbunt* [6].

For scientific needed, the natural medicinal products need to be tested for their chemical content or active ingredients. Bioassay is part of important research to determine the efficacy of herbal drugs. Characterization, isolation, and purification of compounds are needed in the process of development [7].

The purpose of this study was to determine ethnobotany, phytochemical, toxicity, and antioxidant activity from ten medicinal plants species from North Sumatra.

2. Material and methods

2.1. Materials

The samples were obtained from the forest surrounding Purba Tua Village, Borbor District, Toba Samosir Regency, North Sumatra. The research location is at an altitude of 1256 meters above sea level.

2.2. Methods

2.2.1. Ethnobotany study. The study was conducted through in-depth interviews with the communities (local villagers) around the forest regarding the plant species, which they alleged efficacious as medicinal plants, and how they utilized those medicinal plants from the forest.

2.2.2. Extraction. The research materials included the plants or their tree parts that were presumed efficacious as medicine (i.e., bark, fruit, leaves, and roots). Then several pieces from those were taken as the representative material samples. Each of the samples was collected, which was weighed approximately two kg. The sample was dried in the oven at a temperature of 50°C.

The stages of extraction are as follows. Firstly, the samples were ground to powder using a milling machine. Then the resulting powder was strained using a multistage screen to obtain the strained powder with a specific dimension (i.e., 40-60 mesh in size). Secondly, the obtained 40-60 mesh-sized powder was extracted with 96% methanol applying the so-called maceration technique at room temperature for four times 24 hour. Afterwards, the migrated filtrate solution that contained the extract and solvent (methanol) was collected. Finally, the methanol extracts were obtained by separating them from the filtrate solution using a rotary vacuum evaporator at 54°C (in the ultimate dried solid form).

2.2.3. Phytochemical testing. Phytochemical testing was carried out on the dried methanol extracts to identify the extract constituents using standard procedures. The constituents (compounds) which were tested included flavonoids, tannins, saponins, triterpenoids, steroids, hydroquinones, and alkaloids [8].

2.2.4. Toxicity testing. The solid methanol extracts (as previously obtained using rotary vacuum evaporator) were then prepared in aqueous solution form with concentrations of 5000 ppm by dissolving each into the distilled water and afterward diluted further also with distilled water to consecutively 500, 100, 50, and ultimately 10 ppm. If the extract was not soluble in water, dimethylsulphoxide (DMSO) should be added. Next, the seawater in the amount of 400 mL was prepared. Then as much as 600 μL of seawater was taken. Into the 600 μL-seawater was then added as many as 10 shrimp larvae, and then also added 1 mL of aqueous methanol extract (with varying concentrations of consecutively 500, 100, 50, and 10 ppm). Further, the mixture of 600 μL-seawater (that contained 10 shrimp larvae) and 1 mL-methanol extract was then put into the multi-well.
Afterward, the multi-well was covered with a thin aluminum sheet and incubated for 24 hours. In each concentration of the extract could then be calculated the average percentage (%) of dead shrimp larvae (larva mortality).

A chemical substance is said to be toxic to the shrimp (*Artemia salina*) larvae, if it affords LC$_{50}$ (lethal concentration for 50% mortality of the shrimp) value less than (<) 1000 μg/ml (ppm)[9].

2.2.5 Antioxidant testing. The free radical scavenging activity of the extract was based on the scavenging activity of Table 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical that was determined by Blois method (1958) [10]. The crude extract was made in many concentrations (5, 7.5, 10, 15, 25, 50, and 75 ppm). Each extract was put into tubes with 500 µl condensations DPPH 1mM in methanol was added. The volume was made sufficient until 5 ml and was then incubated at 37°C temperatures for 30 minutes, and absorption was measured with a UV-VIS spectrophotometer at 517 nm wavelength. The IC$_{50}$ value was calculated by using the formula equation of regression and was conducted in three replications.

2.2.6. Analysis of chemical compounds. Methanol extracts from those several medicinal plant species were used as material for the analysis of their chemical compounds. The analysis used a Gas Chromatography-Mass Spectra (GCMS) device, located at the Forensic Laboratory Center (Puslabfor POLRI), Police Headquarters. The GCMS specification is an electron ionization (EI) detector attached to the GC-17A gas chromatograph (Shimadzu), which was combined with MSQP5050A mass spectrometer and the database Wiley 7N2008. The column used was an HP 5 MS with an initial diameter (ID) of 0.25 mm and a thickness of 0.25 mm film. The GC/MS instrument (Shimadzu QP2010) run time was set at 39.67 minutes, the initial GC oven temperature at 70°C, the final temperature at 290°C, and used DB5MS detector.

3. Results and discussion

3.1. Ethnobotany study

Ethnobotany information is beneficial in the development of herbal medicines. There are ten species of medicinal plants that are very dominantly used by the local community (Table 1).

| No. | Species                                      | Part of use | Reported activity based on villagers          |
|-----|----------------------------------------------|-------------|----------------------------------------------|
| 1   | *Baccaurea tetrandra* (Baill.) Müll. Arg.   | Bark        | Stomach ulcers                              |
| 2   | *Cinnamomum iners* Reinw. Ex Blume          | Roots       | Medicine for back pain, diuretic             |
| 3   | *Garcinia lateriflora* Blume                | Bark        | Stomach ulcers                              |
| 4   | *Hymenophyllum* sp.                         | Leaves      | Fever medicine                              |
| 5   | *Urena lobata*                              | Leaves      | Fractures medicine                          |
| 6   | *Centella asiatica* (L.) Urb                | Leaves      | Stomach ache                                |
| 7   | *Gaulteria leucocarpa* (1)                   | Leaves      | Fever medicine                              |
| 8   | *Gaulteria leucocarpa* (2)                   | Leaves      | Fever medicine                              |
| 9   | *Saurauia bracteosa* DC.                    | Leaves/ fruit| Antidiabetic                                |
| 10  | *Lycopodium clavatum* L.                    | Leaves      | Antidiabetic                                |

*S. bracteosa* is the most widely used plant species by villagers. The local people use their plant for diabetic treatment and digestive problem. Karo’s people (one of Batak sub-ethnic) said the plant as “cep-cepant lembu.” They have local wisdom in using woody plant species to cure diabetes. Dried leaves (5-8 pieces) are boiled in 1 litter of water until half part remains and then consumed twice up to three times a day [11]. Furthermore, *Saurauia vulcanii* (in one genus) leaves are used for diarrhea,
gastrointestinal disorders, and injury in ethnomedicine of sub-ethnic Batak Simalungun, North Sumatra Province, Indonesia [12].

3.2. Phytochemical content

Phytochemical testing results from medicinal plant extracts are presented in Table 2. Results showed that those medicinal plants generally contained extract contains flavonoids, saponins, tanins, and alkaloids.

| No | Extract                                    | T  | S  | F  | S/T | Alkaloids |
|----|--------------------------------------------|----|----|----|-----|-----------|
| 1  | *Baccaurea tetrandra*(Baill.) Müll. Arg.    | ++ | ++ | +  | +   | ++        |
| 2  | *Cinnamomum iners*Reinw. Ex Blume          | +++| +  | -  | ++  | -         |
| 3  | *Garcinia lateriflora* Blume               | +  | ++ | +  | -   | ++        |
| 4  | *Hymenophyllum* sp.                        | ++ | ++ | +  | -   | ++        |
| 5  | *Urena lobata*                             | ++ | ++ | +  | -   | ++        |
| 6  | *Centella asiatica* (L.) Urb               | +++| +++| +  | -   | -         |
| 7  | *Gaulteria leucocarpa* (1) Urb             | +++| +++| +  | -   | -         |
| 8  | *Gaulteria leucocarpa* (2)                 | +++| +++| +  | +   | -         |
| 9  | *Saurauia bracteosa* DC.                   | +++| +++| +  | -   | -         |
| 10 | *Lycopodium clavatum* L.                   | +  | +  | ++ | -   | ++        |

Ref.: T=tanin; S=Saponins; F=Flavonoids; S/T=Steroids/Triterpenoids; D=Dragendorf; M=Meyer; W=Wagner; H=Hydroquinones; Intensity +++ = strong detection; ++ = medium detection; + = small detection; - = not detection

The phytochemical test of the hydroquinones compound showed that only *Hymenophyllum* sp. contained hydroquinones compound. Hydroquinones are a whitening agent that is most often used for the treatment of pigmentation cases such as dull skin and spots[13]. Thus, these plant species have the potential as cosmetic ingredients, especially skin whitening drugs, removal of spots, and others. The phytochemical screening on *Cichorium intybus* L. hydroalcoholic extract confirmed the presence of tannins, saponins, and flavonoids [14].

3.3. Toxicity activity

Brine Shrimp Lethality Test (BSLT) is one of the screening methods to determine the toxicity of an extract or compound. *Artemia salina* Leach's death is used as a parameter to indicate the presence of cytotoxic active plant substances. This method is also often correlated with the potential for anti-cancer extracts. The results of the toxicity testing of 10 species of medicinal plants, which are shown with LC\(_{50}\) values, are presented in Table 3.

A substance is said to be active or toxic if the LC\(_{50}\) value is <1,000 ppm for extracts and <30 ppm for a compound. Meanwhile, if the LC\(_{50}\) value is > 1,000 ppm, it is not toxic [9]. The results of the toxicity test of all medicinal plants are classified as toxic and have the potential as raw materials for medicines.

Phytochemical research and Brine Shrimp Lethality Test on *S. bracteosa* DC leaf extract have been reported. Leaf extract was further tested to phenolic, flavonoids, and tannins contents. The results showed that all tested samples had a toxicity below 1000 ppm, so they were classified as a toxic substance. The other research, *Saurauia cauliflora* leaf extract, has LC\(_{50}\)= 35.4 ppm. The content of phenolic, flavonoids and tannins compounds was 128 ppm, 44.4 ppm, and 86.75 ppm, respectively [15]. Furthermore, research on Pletekan (*Ruellia tuberosa* L.) leaf extract showed that the LC\(_{50}\) value of successive LC\(_{50}\) values of n-hexane extract was 1389.31 μg / mL, the LC\(_{50}\) value of ethyl acetate extract 453,941 μg / mL and the LC\(_{50}\) value of ethanol extract was 142,160 μg / mL [16].
Table 3. Toxicity activity.

| No | Extract                                    | LC<sub>50</sub>, ppm |
|----|--------------------------------------------|----------------------|
| 1  | Baccaurea tetrandra (Baill.) Müll. Arg.    | 416.81               |
| 2  | Cinnamomum iners Reinw. Ex Blume           | 351.69               |
| 3  | Garcinia lateriflora Blume                 | 663.33               |
| 4  | Hymenophyllum sp.                          | 721.86               |
| 5  | Urena lobata                               | 784.44               |
| 6  | Centella asiatica (L.) Urb                 | 426.99               |
| 7  | Gaulteria leucocarpa (1)                   | 721.86               |
| 8  | Gaulteria leucocarpa (2)                   | 784.44               |
| 9  | Saurauia bracteosa DC.                     | 426.99               |
| 10 | Lycopodium clavatum L.                     | 864.84               |

In vivo Brine Shrimp lethal active compounds isolated from Saurauia roxburghii leaves have been reported. LC<sub>50</sub> data were for various extracts, column fractions, three pure compounds of Saurauia roxburghii, and vincristine sulfate. The crude extracts and isolated pure compounds exposed the potential cytotoxic activities, which may provide support for some of the uses in ethno medicine [17].

3.4. Antioxidant activity

Antioxidant compounds found in plants such as phenolic acids, flavonoids, tocopherol, and tannins are derived from various parts of plants such as leaves, roots, stems, seeds, and flowers. Antioxidants protect the body from free radicals and neutralize them because our body naturally produces antioxidant compounds. However, an antioxidant produced by the body is not strong enough to fight free radicals [18]. The antioxidant activity of ten species of medicinal plants is presented in Table 4.

Table 4. Antioxidant activity.

| No. | Species                        | IC<sub>50</sub> |
|-----|--------------------------------|----------------|
| 1   | Baccaurea tetrandra (Baill.) Müll. Arg. | 27.06          |
| 2   | Cinnamomum iners Reinw. Ex Blume | 104.81         |
| 3   | Garcinia lateriflora Blume     | 35.38          |
| 4   | Hymenophyllum sp.              | 45.70          |
| 5   | Urena lobata                   | 207.63         |
| 6   | Centella asiatica (L.) Urb    | 58.97          |
| 7   | Gaulteria leucocarpa (1)       | 53.19          |
| 8   | Gaulteria leucocarpa (2)       | 41.08          |
| 9   | Saurauia bracteosa DC.         | 146.40         |
| 10  | Lycopodium clavatum L.         | >250           |

The range of a compound is classified to have a very strong antioxidant having an IC<sub>50</sub> value <50 ppm, an IC<sub>50</sub> strong group between 50-100 ppm, a moderate group 101-150 ppm, and a weak group having an IC<sub>50</sub> value between 150-200 ppm [19]. In general, the antioxidant activity of ten medicinal plant species studied had good activity. There are many reported antioxidant activities on the medicinal plants from the forest. Antioxidant activity of the water extract of S. vulcani IC<sub>50</sub> values is 22.92±1.32μg/mL, whereas IC<sub>50</sub> produces quercetin value of 4.96±0.02 μg/mL. These results show that the ability to capture free radicals of the S. vulcani water leaves extract includes a strong antioxidant [20].

The antioxidant activity (IC<sub>50</sub>) value of Cichorium intybus L. hydroalcoholic extract resulted by DPPH method was found to be 67.2±2.6μg/ml [14].

The other research on Pteridium aquilinum Kuhn leaves extract contains a good amount of antioxidant activity, which is a comparison with ascorbic acid. With DPPH, radical scavenging
activity method results in IC$_{50}$=88.00 ppm, and with ABTS, radical scavenging activity method results in IC$_{50}$= 73.33 ppm [21].

### Table 5. Chemical compound.

| No | Species                      | Component                                                                 | %   |
|----|------------------------------|---------------------------------------------------------------------------|-----|
| 1  | Baccaurea tetrandra (Baill.) Müll. Arg. | Gamma sitosterol, Hexadecanoic acid, Erucyl amide, Octadecanoic acid, 3-nitro-O-methylateroline | 20.81 |
| 2  | Cinnamomum iners Reinw. Ex Blume | Hexadecanoic acid, Neophytadiene, Gamma sitosterol, Phytol, Octadecanoic acid | 6.15 |
| 3  | Garcinia lateriflora Blume  | 1,4-bis(Trifluoromethyl)-2,4,9,10-tetrahydro Critoxy arboreno E, Gamma sitosterol, Alpha tocopherol, Linoleic acid | 23.69 |
| 4  | Hymenophyllum sp.            | Linoeleic acid, Hexadecanoic acid, Stigmaster-5-en-3-ol, (3.beta.), Neophytadiene, Alpha tocopherol | 10.41 |
| 5  | Urena lobata                 | Benzoic acid, 2-hydroxy-, methyl ester, Hexadecanoic acid, 5-Hydroxymethylfurfura, Palmitad acid, Linoleic acid | 31.21 |
| 6  | Centella asiatica (L.) Urb  | Hexadecanoic acid, Gamma sitosterol, caratenoid, Asiticoside, Linoleic acid | 17.27 |
| 7  | Gaulteria leucocarpa (1)     | Benzoic acid, 2-hydroxy-, methyl ester, Squalene, 5-Hydroxymethylfurfura, Palmitad acid, Linoleic acid | 20.40 |
| 8  | Gaulteria leucocarpa (2)     | Benzoic acid, 2-hydroxy-, methyl ester, Squalene, 5-Hydroxymethylfurfura, Palmitad acid, Linoleic acid | 31.21 |
| 9  | Sauraria bracteosa DC.       | Hexadecanoic acid, Gamma sitosterol, alpha.-Amyrin, 3-KETO-URS-12-ENE, Linoleic acid | 17.27 |
| 10 | Lycopodium clavatum L.       | Hexadecanoicacis, Lycopodin, Oleic Acid, Stigmasterol, Octadecanoic acid | 26.21 |
Furthermore, extract yield, the chemical composition of the *Withania somnifera* extracts, and antioxidant activity of the extracts varied with the extraction process as well as solvent composition. Antioxidant capacities of the extracts were expressed in terms of IC\(_{50}\) value of the extracts, and low IC\(_{50}\) value corresponded to a high antioxidant capacity. In both DPPH and ABTS assays, ethanol extracts had the lowest IC\(_{50}\) values, and they varied in the following order: ethanol < water – ethanol < water [22].

3.5. Chemical compound
The main chemical components of ten species of medicinal plants based on GCMS analysis are presented in Table 5.

Alpha-tocopherol is a compound that has Vitamin E activity. Its main benefits are antioxidants and immune system stimulation. Increased immunology is associated with increased immunity [23]. Likewise, the gamma sitosterol compound and other sitosterol groups are compounds that are both antioxidants and anticholesterol.

Hydroxyl acids, such as exadecanoic acid, octadecenoic acid, and oleic acid, are known to have activity in microbial inhibition [24].

4. Conclusion
There are about 30 species of medicinal plants used by villagers. The local people used ten main species of natural medicine. The medicinal plants are used for various diseases or disorders such as stomach ache, fever medicine, fractures medicine, stomach ulcers, diuretic and antidiabetic. The phytochemical testing showed that the extract contained flavonoids, saponins, tannins, and alkaloids. All medicinal plants are classified as toxic based on toxicity testing and have the potential as raw materials for medicines. The antioxidant activity (IC\(_{50}\)) were Sikkam 27,06 ppm; Modang Kulim 104,81 ppm; Handis 35,38 ppm; Rugi-rugi 45,70 ppm; Sambang-sambang 207,63 ppm; Ampapaga 58,97 ppm; Sae-sae putih 53,19 ppm; Sae-sae hitam 41,08 ppm; Pirdot 146,40 ppm and Sirungguk more than 250 ppm. In general, the antioxidant activity of ten medicinal plant species studied had good activity. These plant species have the potential to be developed into standardized herbal medicines.

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