Toxicity and Total Phenolic Content of *Saurauia vulcani* Extracts from Cultivation

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Abstract. *Saurauia vulcanii* plant has been widely used to treat a variety of diseases suffered by villagers as antidiabetic and digestive problems’ remedy. The Forestry Research and Development Agency has cultivated this plant at Forest Area for Specific Purpose in Northern Sumatra. The aims of this study were to determine the toxicity and total phenolic content from *n*-hexane, ethyl acetate and methanol *Saurauia vulcani* extracts. The toxicity was carried out using the Brine Shrimp Lethality Test (BSLT) method and Folin Ciocalteu method is used for total phenolic content. The result showed that the toxicity of *n*-hexane, ethyl acetate and methanol extracts i.e. LC50 values of 365.19 ppm, 715.28 and 225.77 ppm, respectively. The total phenolic content of hexane, ethyl acetate and methanol extracts were 7,155 mg GAE/g, 13,702 mg GAE/g and 16,560 mg GAE/g, respectively.

1. Introduction
Plants of the genus *Saurauia* are widely distributed in Indonesia. *Saurauia vulcani* is one of species that is quite widely distributed. This species grows widely in the Lake Toba’s Catchment Area, located in North Sumatra, Indonesia. The spread of this species is found in several areas in North Sumatra such as in Toba, North Tapanuli, Simalungun and Tana Karo districts. This simplicia has been widely trafficked on the roads across Sumatra with claims to be anti-diabetic herbs. The Tapanuli villagers said this plant as *pirdot* which is believed able to treat various diseases related to the digestive system.

*Saurauia vulcani* leave is one of woody plant species have been used to cure diabetes at Karo’s local wisdom. Situmorang et al. said that the in practice of diabetes treatment is done by boiling 5-8 dry leaves, then boiling with a liter of water to leave half of the water. This herb is consumed 2-3 times a day.[1] Traditionally, the people of the Batak Simalungun sub-ethnic group, North Sumatra Province, have used parts of *Saurauia vulcanii* leaves for treatment such as diarrhea, gastrointestinal disorders, and wounds.[2]

Research conducted by Sitorus showed that the characteristics of the ethanol extract of *Saurauia vulcani* showed 6.65% and 7.25% in water content. Meanwhile the water soluble extract values were 23.55% and 64.25%.[3]

Research on antioxidants has been carried out on the aqueous extract of the *Saurauia vulcani* plant, where the IC50 value is 22.9182 ± 1.32 ppm while the IC50 quercetin is 4.96 ± 0.02 ppm. The results of this study indicate that *Saurauia vulcani* has a strong antioxidant. This is based on the strong category of antioxidants with a concentration value of 50-100 μg / mL.[4]
There are several chemical compounds that have been isolated from the genus Saurauia, namely 3β-hydroxy-Olean-12-en-28-oat acid; 3,19-dihydroxyurs-12-en-28-oic acid; some monoterpene lactones; triterpenoids, fatty acids; and two steroids, stigmasterol; and β-sitosterol.[5][6][7]. There are several biological activities afforded by Saurauia vulcani i.e. antioxidant activity, anti-cholesterol, antidiabetic activity, antihyperlipidemic, analgetic activity, antimicrobial activity, and wound-healing activity.[8][9][10][11]

Research on Saurauia plants usually uses wild plants as research objects. In this study, the material plant used is the result of cultivation at the age of 4 years. This research will investigate how the differences in secondary metabolites produced from wild plants and cultivated plants. The brine shrimp lethality test value has correlated with cytotoxicity activity in plant extracts. Simultaneous bioassay testing can be used simultaneously to test various natural marine products for pharmacological activity.[12]. Furthermore, the total phenolic content can predict the antioxidant activity of the extract. Polyphenols have properties in preventing oxidative stress associated with cancer. Further, polyphenols also have other biological activities in prevention and treatment.[13]

The aims of this study were to determine the toxicity and total phenolic content from n-hexane, ethyl acetate and methanol Saurauia vulcani extracts.

2. Experimental details

2.1. Plant material
Saurauia vulcani was collected from Sipiso-Piso, Tana Karo Regency, at Forest Area for Specific Purpose in Northern Sumatra. The collected plant material was air-dried and powdered.

2.2. Extraction
Dried ground leaves (1 kg) of Pirdot plant (Saurauia vulcani) leaf and flowers were extracted with multilevel extraction method by used three solvents i.e. n-hexane, ethyl acetate and methanol. The filtrated obtained is concentrated using a rotary vacuum evaporator.

2.3. Brine Shrimp Lethality Test
The fractions of n-hexane, ethyl acetate and metanol were evaluated in a test for lethality to brine shrimp larvae. Toxicities of compounds were tested at 1, 10, 100, and 1000 ppm in 10 mL sea-water solutions with 1% DMSO (v/v). In this test ten larvae were used in each test and counted how many were alive after 24 hours. At each concentration 3 replications were set. Distilled water is used as a control blank. The lethality concentration (LC50) was calculated using probit analysis. The LC50 value is calculated using a regression line obtained by plotting the concentration against the percentage of mortality on a probit scale.[14]

2.4. Total phenolic contents in Saurauis vulcanii extracts
The spectrophotometric method can be used to measure phenol content in plant extracts.[15]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of solution of extract, 2.5 ml of 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. The samples were there after incubated in a thermostat at 45 °C for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure is performed on the standard solution of gallic acid and the calibration line. The measured absorbance value, the phenolic
concentration can be read from the calibration line. The phenolic content in the extract is expressed as mg of gallic acid equivalent (mg GA/g of extract).

3. Result and Discussion

3.1. Toxicity Activity

Brine Shrimp Lethality Assay is a fairly practical method for initial screening to determine the toxicity of plant extracts. The advantages of this method are fast and simple. However, it is necessary to control the research conditions such as temperature, pH, salinity, aeration and lighting. The concentrations used in this study were in the range of 10, 100, and 1000 µg/ml. In general, the determination of toxicity is determined after 24 hours.[16]

The results of the toxicity test on n-hexane, ethyl acetate and methanol extracts i.e. LC50 values of 365.19 ppm, 715.28 and 225.77 ppm, respectively. Toxicity value is classified as non toxic if the LC50 more than 1000 ppm. The low toxic is classified if the LC50 from 500 to 1000 ppm. The medium toxic is classified if the LC50 from 100 to 500 ppm. The high toxic is classified if the LC50 from 0 to 100 ppm.[17]

Hamidi et al., reported the LC50 values from ethanol extract of suren seeds, mahogany, mimba, and saga were 75, 84, 323 and 449 ppm, respectively. The four extracts are classified from medium toxic to high toxic.[18]. Based on Albuntana et.al., stated that Bohadshia argus extract has the active species indicated by LC50 value 69,254 ppm from their research. Furthermore, water fraction of Holothuria leucospilota’s crude extract is the most active fraction indicated by LC50 at 50,968 ppm.[19]

The toxicity activity of some plant in Meliaceae famil have been reported i.e Azedirachta indica, Azediraeha indica var. siamensis, Melia azedarach, Sandoricum indicum and Swietenia macrophylla species. The toxicity of stem bark extracts showed the significant toxicity from 8.63 to 234.06 ppm, whereas part of leaves were inactive. Researcher reported that the leaves and seed part have the higher potency than the stem bark part. [14]

The LC50 of Annona reticulata with Allium fistolisum and Brassica oleracea alcoholic extract were 24.162, 13.433 and 10.818 ppm, respectively. The toxicity of those plant is classified as high toxic and potential as anticancer agent.[20]

Based on Puspitasari that the leaves extract Avicennia marina, Rhizophora mucronata and Sonneratia alba have the LC50 i.e 403.44 ppm, 709.7 and 801.75 ppm, respectively. The Avicennia marina leaf extract had a potential to be the strongest toxic among other extracts.[21]

Innocent et al., have reported the results of a toxicity study on 30 medicinal plants in the Bukoba district, northwestern Tanzania. The solvents used are dichloromethane and/or ethanol. The toxicity of the ethanol extract of Lantanta trifolia (LC50 32.3 ppm), Vernonia bradycalyx (LC50 33.9 ppm), Aniarias toxicaria (LC50 38.2 ppm) and Rubus rigidus (LC50 41.7 ppm) and dichloromethane extract from Gynura skandens (LC50 36.5 ppm) and Bridelia micrantha (LC50 32.0 ppm) and categorized as mild toxic. Meanwhile, the dichloromethane extracts of Picralima nitide (LC50 18.3 ppm) and Rubus rigidus (LC50 19.8 ppm) were classified as quite toxic. Only 1.1 and 1.2 Picralima nitida and Rubus rigidus extracts were classified as less toxic when compared to the standard drug, cyclophosphamide (LC50 16.3 ppm) [22].

3.2. Total Phenolic Content of Saurauia vulcani

The total phenolic content of Saurauia vulcani are 7.155 mg GAE/g, 13.702 mg GAE/g and 16.560 mg GAE/g of dry weight of extract, respectively for hexane, ethyl acetate, and methanol extract. This value indicates that in every gram of extracts of n-hexane, ethyl acetate and methanol, the equivalent of 7.155, 13.702 and 16.560 mg of gallic acid.

Lovenia et.al., reports that the antioxidant activity of Saurauia vulcani from wild plant is strong activity [4]. The value of total phenolic content can be used as approach to predict the antioxidant activity. The relationship between total phenolic content of noni leaf extract and antioxidant activity has
a coefficient of determination of 0.9820 [23]. Trisharyanti et al. said that phenolic content and antioxidant activity of ethanol extract Cashew leaves showed a positive correlation of 58.8% [24].

When compared with the results of total phenolic content in wild and cultivated Saurauia vulcanii, it shows different results. Total phenolic content of plant from cultivation is tendency smaller than from wild. This is probably influenced by the age of the plant which is still quite young (4 years). In general, the Saurauia vulcanii plant taken from nature is a tree that is mature and bigger. Total phenols, flavonoids, saponins, alkaloids, triterpenes and steroids increased with age of the Neonomotia wightii tree. [25]

Almost the same results were reported by Hossain and Shah with this study. They conducted research on total phenolic content in the Merremia borneensis plant. The total phenol content of hexane, ethyl acetate, chloroform, butanol and ethanol extracts were 5.90, 6.68, 20.25, 6.84 and 30.35 GAE/g, respectively [26].

Different study results are reported by Herawati on mangrove plants used hexane, chloroform, ethyl acetate and methanol as solvents. The results showed that the total phenolic content of the three fractions (hexane, chloroform, ethyl acetate) and the methanol extract of Sonneratia alba’s bark were 17.84 ± 0.974, 156.310 ± 0.703, 226.89 ± 0.605, 249.56 ± 0942 respectively. GAE μg/g [27]. A corresponding study was carried out by Semiring et al that reported variations in the total phenolic content of surian (Toona sinensis) leaves. The results showed that the total phenolic content varied from 276.62 to 444.68 (mg GAE/g). [28]. While the four crude extracts of Caesalpinia bonduc (L.) Roxb, the leaves contained the highest amount (31.05 ± 0.35 mgQE / g). [29]

Research on total phenolic content is not carried out only on medicinal plants. Total phenolic content is also carried out in vegetable and fruit crops. The vegetables and fruit that were tested were the result of planting in the Tower Garden aeroponic system and in the ground. The results showed that the vegetables that had the highest phenol content were chaletard 57.73 mgGAE/g in aeroponic growing system and 53.45 GAE/g in the field followed by basil, red cabbage, and parsley. This study showed that vegetables grown in soil had a slightly higher phenolic content in basil, turnips, and parsley than those grown in an aeroponic system. Meanwhile, the total phenolic content, red kale is slightly higher in aeroponic [30].

Furthermore, Aryal et al reported the results of a study on phenolic content in eight selected wild vegetables from Nepal (Alternanthera sessilis, Basella alba, Cassia tora, Digera muricata, Ipomoea aquatica, Leucas cephalotes, Portulaca oleracea and Solanum nigrum). This research uses methanol as a solvent in its extraction. The phenolic compound content ranges from 292.65 to 72.66 mg GAE/g. Vegetables Alternanthera sessilis, Cassia tora and Portulaca oleracea had the largest phenolic content (292.65 ± 0.42, 287.73 ± 0.16 and 216.96 ± 0.87 mg GAE/g, respectively). Meanwhile, the smallest phenolic content was found in Basella alba, Ipomoea aquatica, and Solanum nigrum (72.66 ± 0.46, 77.06 ± 0.70 and 97.96 ± 0.62 mg GAE /g respectively).

Stankovi reported there was a significant correlation between the total phenolic content and the antioxidant activity of the plant extracts studied. Researcher reported that five different extracts of the entire herb Marrubium peregrinum L. (Lamiaceae). The results of the spectrometric study showed that the total phenolic content of the extract ranged from 27.26 to 89.78 GAE/g. Researchers also proved that the higher the phenolic compound content, the higher the antioxidant activity. [22]

There are several things that affect the total phenol content. The total phenol content was studied in terms of susceptibility to degradation of aqueous solutions in plant extracts, the effect of light, during storage at room temperature for fortification of food products. The total phenol content in the model solution (olive leaf, green tea, red wine, red wine, 5: 1 PE pine bark, 95% PE pine bark, resveratrol), ranged from 11.10 mg GAE/100 mL to 92.19 mg GAE/100 mL [31].

Noreen et al., Reported variations in the total phenolic content in different solvents. The highest total phenolic content was found in the ethanol extract of Coronopus didymus (47.8 mM GAE) and the lowest in the dichloromethane extract (3.13 mM GAE). Furthermore, using a calibration curve, ethanol extract
showed the highest phenolic content (47.8 mM GAE) followed by acetone (38.6 mM GAE) and n-hexane (27.2 mM GAE) while chloroform and dichloromethane extracts showed the lowest. The total phenolic content variation reaches 15 times [32]. The polarity of solvent used in extraction can affect the total phenolic contents in plant extracts. In polar solvents have a high solubility of phenols will provide a high concentration.

4. Summary
The results of the toxicity study showed that the hexane and methanol Saurauia vulcani extracts were classified as moderate toxic, while the ethyl acetate extract was classified as low toxic. Meanwhile, the total phenolic content of hexane, ethyl acetate and methanol extracts were 7,155 mg GAE/g, 13,702 mg GAE/g and 16,560 mg GAE/g, respectively.

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