Compound Content of Local Curcumin (Curcuma Xanthorrhiza) in North Sulawesi, Indonesia

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Abstract
This Zingiberaceae plant is widely used as medicine, including temulawak. Because curcuma (C.xanthorrhiza) is a medicinal plant that has many benefits and includes plants needed in large quantities compared to other medicinal plants. Traditionally rhizomes Temulawak is used to treat stomach ailments, liver disorders, constipation, diarrhea, dysentery, fever, hemorrhoids, hypotriglyceridaemic, and anti-inflammatory. Study about plant Curcuma local ignite still not enough researched so that not yet get information compounds which contained in the local curcuma of North Sulawesi for the development for biopharmaceutical development, mapping in the distribution of plants, ecology, plant conservation and protection to maintain local wisdom. Histochemical Test is a method to determine the content of chemical compounds in a plant tissue qualitative. Testing can be done by adding a special reagent or solution to the incision organ plant and will give color which Specific. Activity study this will test curcumin compound group on local ginger from North Sulawesi by histochemical method. research that done is study non experiment with design descriptive qualitative. The results showed that North Sulawesi local white temulawak powder contained curcumin in a sample with a sample weight of 0.10 g at a sample spotting volume of 20 µl with a sample spotting volume of 2040 nanograms, curcumin levels were <0.10 nanograms/mg. Curcumin biosynthesis is influenced by site conditions, agro-climate, genotype, and plant cultivation.

Keywords— North Sulawesi Local Curcuma (C. Xanthorrhiza), Curcumin Compound, Histochemical Test

Introduction
Indonesia is a tropical country which is famous for its natural wealth. One of natural wealth owned in the form of diversity of plants that can be used as plants ornamental, vegetables and most importantly can be used as medicines that are useful for health and efficacious [1]. One of them is the ginger plant, and the part that is often used of this plant is the rhizome. Temulawak rhizome contains high antioxidants and can be used as a traditional drink known as herbal medicine [2]. Consuming ginger on people healthy also very urgent especially for look after health function heart and guard body immunity. In the midst of the Covid-19 pandemic, it is important to maintain the body's immune system by consuming it adequate nutrition and regular rest [3].

As is known that with the pandemic Covid-19 Indonesian people, especially the people
of North Sulawesi, are experiencing an economic decline or reduced income so that they cannot
buy drugs that are classified as expensive, but the people of North Sulawesi can consume
traditional medicines that are around us who can afford it increase the body’s immunity by
consuming herbal medicine because one of the recipes for making herbal medicine Derived from
the ginger plant and herbal medicine, it contains natural ingredients in the form of part of plant
like ginger rhizome. Plant Zingiberaceae this many used as drug, among them ginger [4].

Literature Review

Curcuma (C. xanthorrhiza) is wrong one plant drug which many the benefits and
including plant which needed in total big compared plant drug others [5]. Kindly traditional
rhizome Curcuma used for treat disease stomach, disturbance heart, constipation, diarrhea,
dysentery, fever, hemorrhoids, hypotriglyceridaemic, and anti-inflammatory [2][5]. Curcuma
plants grow well and can adapt in the open or under stands trees to a shade level of 40% [9]. The
average national production is relatively low, namely 10.7 ton/ha in year 2003, whereas potency
production varieties superior Curcuma can reach 20 - 30 n/ha [7] [10]. There is no research on
histochemical tests of tissue or cells of local temulawak rhizome in North Sulawesi been carried
out, to determine the compound curcumin found in local temulawak plants North Sulawesi, given
the benefits and high market value of commodities, it needs to be developed. Specifically North
Sulawesi local temulawak can improve the standard of living of farmers and the community
because it opens up opportunity center beverage and drug industry.

Research Method

The preparation of plant material comes from the local temulawak plant of North
Sulawesi. Other materials consist of 5% CuSO4, 70% alcohol, 85%, 95%, 5% AlCl3, 30%
glycerin, Wagner reagent, Sudan III 0.03%, FeCl3, and NaCO3. The tool used is the Olympus
microscope DP73 with lab optical camera and microscope, petri dish, cover glass, object glass,
dropper pipette, tweezers, beaker glass, measuring cup, measuring flask, waterbath, and razor
blade. The research conducted was non-experimental research with a descriptive design. Data
which obtained were analyzed qualitative descriptive. Study this done March – October 2022
During 8 month in the field (in vivo) and laboratory (in vitro) . This research was conducted at
the Tomohon Plantation, North Sulawesi, Indonesia. Analysis compound curcinoids based on
the test histochemistry was carried out at the Integrated Research and Testing Laboratory (LPTP)
Gadjah Mada University (UGM) Yogyakarta, Indonesia.

Observation of secondary metabolites of curcumin compounds with histochemical tests
was carried out by provide different reagents or solutions according to the test of the compound,
next observation microscopic compound metabolites with some test histochemistry. rhizome
Curcuma made extract with grind until fine, Then done test histochemistry of several secondary
metabolites. The scour results were tested with several kinds reagent. Testing Compound
curcin on cell or network done with gift reagent CuSO4 5%. The presence of curcumin
compounds will be indicated by differences in color and the results must be positive curcumin.
Stages study : 1) Selection sample plant which will extracted, 2) Manufacturing reagent, 3)
Optimization preparation samples, 4) Preparation Sample, 5) Determination rate curcumin in
samples, 5) Data analysis. Data were analyzed based on the formulation [6]:

\[
\text{Curcumin sample (ng)} = \frac{(\text{Sample area} - b)}{a}/1000
\]

\[
\text{Curcumin level (}\% \text{w/w)} = \frac{(\text{Curcumin level/µg})}{\text{spotting sample (µg)}} \times 100
\]
Results and Discussion

Table 1. Curcumin Levels in North Sulawesi Local White Temulawak Material Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Weight (g)</th>
<th>Sample spotting volume (μl)</th>
<th>Final Additions (ml)</th>
<th>Number of Spottings (ng)</th>
<th>Curcumin in Sample (nanograms)</th>
<th>Curcumin levels (ng/mg)</th>
<th>Average (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcuma powder local white from North Sulawesi</td>
<td>0.1020</td>
<td>20</td>
<td>1</td>
<td>2040.00</td>
<td>5337.29</td>
<td>&lt;13.06</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td></td>
<td>0.1016</td>
<td>20</td>
<td>1</td>
<td>2032.00</td>
<td>5996.01</td>
<td>&lt;13.06</td>
<td>&lt;0.10</td>
</tr>
</tbody>
</table>

Source: Report on test results of the Integrated Research and Testing Laboratory (LPTP) Gadjah Mada University, Yogyakarta, Indonesia (2022)

Figure 1. Plants, rhizomes and powder of North Sulawesi local white curcuma, (2022)

Based on the results of the analysis of local white curcumin powder in North Sulawesi in table 1, as well as the description of the local white curcuma plant in Figure 1, it shows that the curcumin in the sample with a sample weight of 0.10 g at a sample spotting volume of 20 μl with a sample spotting volume of 2040 nanograms obtained curcumin levels <0.10 nanograms/mg. Curcumin biosynthesis is influenced by site conditions, agro-climate, genotype, and plant cultivation. Curcumin is a secondary compound of the phenol group in the Curcuma plant, formed via the shikimic acid and malonic acid pathways from the simple carbohydrate precursors phosphoenol pyruvate and 4 phosphate erythrocytes into aromatic amino acids [6]. Phenylalanine is one of the many aromatic amino acids found in higher plants, this compound is processed into trans-cinnamic acid by eliminating ammonia using a phenylalanine-ammoniac-lyase catalyst, producing simple phenols and complex phenols [6]. The next process produces pkumaryl CoA, which is trans cinnamic acid and further processed into bisdesmethoxy curcumin, desmethoxy curcumin and curcumin. Curcumin is formed from the reaction between p kumaryl CoA and malonyl which is catalyzed by polyketide-synthase enzymes, or the reaction of p kumaryl CoA and Feruloyl CoA with malonyl CoA [8].

The precursors of curcuminoids are ferulic acid and caumaric acid [6]. The curcumin framework can be formed by one of two pathways. The first pathway (a) involves cyclization of a polyketide chain consisting of phenylpropanoid units and five C₂ units either from malonic acid
or via malonyl CoA to produce a second aromatic ring. Pathway (b) involves the condensation of two phenylpropanoids at the central carbon shared by a malonic acid molecule. The biosynthetic pathway of curcumin in temulawak starts from primary and secondary metabolism. The metabolic pathway leading from sucrose to the phenylpropanoids and terpenoids formed in turmeric and ginger can be evaluated at the transcriptional level. These genes can appear if the environmental conditions needed for these genes to form curcuminoids are met [8].

The existence of a determining enzyme in the synthesis of curcumin, due to its position in metabolism in plant tissues is at the junction point of primary and secondary metabolites, in plant tissues these enzymes increase under certain environmental conditions [8]. So that the development of rhizomes and the accumulation of curcumin both depend on the translocation of metabolites formed from the leaves. The amount of metabolites produced by the leaves and the proportion translocated to the rhizomes will greatly affect the size and yield of rhizomes, biosynthesis and accumulation of curcumin. The portion of photosynthate that is translocated to storage organs (rhizomes) is one of the factors controlling productivity, apart from the biosynthetic capacity of the rhizomes [7]. The curcumin biosynthetic pathway in turmeric uses compounds with labeled C atoms, indicating that curcumin is formed from two phenylpropanoid units derived from phenylalanine and one carbon units from malonic acid and phenylalanine combined in the curcumin framework via cinnamic acid [6] [7][8].

Curcumin (diferuloylmethane), obtained from Curcuma aromatica is widely used as a coloring agent and spice in food, has strong anti-inflammatory, antioxidant, and anti-carcinogenic properties [7]. Three important properties of curcumin have been studied with respect to HCC: Anti-HCC; anti-metastatic; and anti-angiogenesis activity [8], by examining the effects of curcumin in a mouse model of HCC; cancer induced by N-diethylnitrosamine (DEN) and reported that curcumin effectively inhibited DEN-induced HCC in C3H/HeN mice. DEN-treated mice showed a remarkable increase in levels of p21 (ras), expression of proliferating cells via the Biomarker of Inflammation: An Important Tool for Herbal Drug Discovery nuclear antigen (PCNA) and CDC2 protein, whereas curcumin reversed the levels of all these biomarkers. The effect of curcumin and tetrahydro curcumin on tumor angiogenesis of HCC mice was also found in a study [7].

The human HCC cell line (HepG2) was inoculated with tetrahydro-curcumin that was orally injected into the back exfoliating chambers of male BALB/v nude mice at 300 and 300 mg/Kg –1 per day. A video microscopy fluorescence and capillary vascularization (CV) were observed to measure tumor microvascularity, with the resultant substantial reductions in curcumin and tetrahydrocurcumin (THC) in CV. THC and curcumin have dose dependent anti-angiogenic effects and are a common mechanism for action against cancer [7].

Conclusion
Based on the results of analysis of local white curcuma powder, North Sulawesi, it was concluded that curcumin in a sample with a sample weight of 0.10 g at a sample spotting volume of 20 µl with a sample spotting volume of 2040 nanograms obtained curcumin levels <0.10 nanograms/mg. Curcumin biosynthesis is influenced by site conditions, agro-climate, genotype, and plant cultivation.

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Disclosure of conflict of interest
Authors declare no conflict of interest.

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