Chemical Profile by LC-MS/MS and Some Bioactivities from Leafs of Kolowe (Chydenanthus excelsus): A Wild and Rare Plant from Indonesia

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Abstract
Secondary metabolites from Kolowe leaf (C. excelsus) with chemical screening are saponins and flavonoids, spread in fraction methanol, ethyl acetate, and a water fraction. Profile secondary metabolites of methanol and ethyl acetate extract from an analysis of LC-MS/MS indicated twenty compounds with molecular masses ranging from 226-1064 for fraction of methanol, suspected of ten types of saponin and compound 10 as flavonoid glycosides. Furthermore, ethyl acetate fraction contained eight compounds with a molecular mass range at 239-1383 consisting of fifty-three compounds saponins and flavonoids. Methanol crude extract, extract a fraction of methanol and ethyl acetate potentially cytotoxic with value LC50 < 50 ppm against A. salina and has an antioxidant activity with value LC50 < 50 ppm against DPPH. In addition crude extract methanol, methanol fraction and the ethyl acetate fraction to be antimicrobial against 19 types of microbes that against gram positive and gram negative bacteria as well as against some fungi.

Keywords: Kolowe leaf (C. excelsus); Chemical profile, cytotoxic, antioxidants, antimicrobials.

INTRODUCTION
Kolowe (C. excelsus) as wild plants and rare in the world, continues to studies of the fruit, leaves, bark, stems, roots, and flowers. The study focused on the phytochemical, biological activity, and domestication. Chemical content useful for uncovering Chemotaxonomic and marker molecules that play a role in biological activity as potential pharmaceutical and ensuring disclosure is considered the commercial potential in the fields of pharmaceuticals and other industries, while the prospect of domestication aiming for cultivation on soil type or different habitats. Although kolowe plants including rare, at Kamaru, Buton, Indonesia still abundance amounts which occupied certain lands wide, used by the community as a source of poison to capture fish in the sea around. The availability of is a potential and further research is required utilization without damaging its sustainability. Kolowe as plant trees, for the sustainable use of scientific consideration, needs for a relatively long life cycle which is 6-10 years [2]. Interest aimed use of non-timber and focused on parts of the plant that does not interfere with the survival of the species although part of the plant used commercially. Utilization of the fruit and leaves kolowe be sustainable though its use on a large scale will not interfere with the survival of plants. Kolowe fruits have proven potential in pharmaceutical field prospect exploited because not interfere with survival [3]. If the leaf has the potential, at least equal to his. Therefore, this study focuses on the benefits of the leaves as an extension of benefits Kolowe plants that will not interfere with continued of life. The traditional use of the Kolowe leaf by the people from Kamaru, Buton, Indonesia limited only as additional materials use the seeds as a fish poison to marine fishing activities. The community considers that leaves Kolowe also has toxins that can increase the toxicity of the seed so that the seed used as an additive for a fish poison. Thus traditionally utilized the same kolowe leaves with seeds but in a smaller size. In science can be mean that the leaves kolowe have the higher toxicity of the seed because only small amounts are needed to increase the toxicity of the seeds to the needs of sea fishing. Accordingly, the content of secondary metabolites and potential pharmaceutical Kolowe leaves very importantly to do well.

MATERIALS AND METHOD
Samples Plants and Materials Research
Leaves of kolowe plant sample which is taken from Kamaru, Buton, Southeast Sulawesi, Indonesia in August 2015. Leaf took from the kolowe tree with a height ranging 3 – 5 m. The samples leaf were immediately sorted and washed with water, then dried until the water content ranges from 10-15%. The dried leaves Kolowe mashed into 3.28 kg dried powder and macerated. Materials needed for extraction and fractionation is 96% methanol; n-hexane, and ethyl acetate, and distilled water.

Extraction and fractionation.
3.28 kg dried powder of Kolowe extracted with methanol. The extract evaporated from the solvent using a rotary evaporator to obtain 645.64 g extract. About 300 g of extract was mixed with 300 g of silica gel GF60 then be included in a Buchner funnel as column chromatography. Fractionation used the technique of solid-liquid, stationary phase silica gel GF60 and solvent or mobile phase n-hexane, ethyl acetate, and methanol. Extract fractions were obtained respectively 2.28 g fraction of n-hexane; 165.6 g ethyl acetate extract fraction, 98.66 g methanol fraction, and the remaining fraction of the water that is not
measured. N-hexane fraction is chlorophyll that does not proceed in the analysis and testing of biological activity.

Profile Analysis of Secondary Metabolites Compounds
Profile analysis of secondary metabolites is determined by chemical methods (reagents) and analysis of LC-MS/MS. Chemistry profile analysis by chemical methods aimed to alkaloids, flavonoids, triterpenes, steroids, phenolics and saponins. The entire metabolite analysis on methanol crude extracts (crude extracts), while the analysis of LC-MS/MS made to extract methanol and ethyl acetate.

a. Secondary Metabolite Screening with Chemical Method
Screening of secondary metabolites kolowe leaf extract made to the crude extract. Each test reagent was added to the extract solutions were prepared. Determination of the class of secondary metabolites contained in the extract is based on the physics of each test changes as a result of chemical reactions between the reagent with chromophore secondary metabolites or the active center of reactive compounds that are identified [4].

b. Analysis of the Secondary Metabolites by LC-MS/MS
UPLC-QToF-MS/MS System (Waters) with software data processing MassLynk version 4.1. Profile of UPLC Acquity SDS (Waters) with column Acquity UPLC BEH C-18 1,7 μm, 2,1 x 50 mm; flow rate 0,3 mL/min; injection 5 μL; temperature 40 °C; eluent water dan 0,1 % formic acid (A); acetonitrile and 0,1 % formic acid (B), eluted by gradient. Extracts have analyzed a fraction of methanol and ethyl acetate.

Biological active assay
Assay conducted for biological activity is cytotoxic activity test, antioxidants, and antimicrobial screening against 25 types of bacteria and fungi. Biological activity test was carried out on the crude extract of methanol, the methanol extract fraction, and ethyl acetate fractions. For cytotoxic and antioxidant tested by storage stability.

a. Cytotoxic activity
Cytotoxic test conducted extract against *A. salina* larvae. Testing methods commonly done is the preparation of the third instar larvae of *A. salina* with hatching eggs *A. salina* techniques for 48 hours. The number of larvae each replication in the test are 10 selected by specific criteria, among others, lively or active. The extract was found for cytotoxic testing shown in Table 1. Concentrations of test series were found by a preliminary test to determine the concentration of the test series. Based on the concentration can be ascertained series LC50 values of each extract of <30 ppm so that it uses the term cytotoxic test.

b. Antioxidant assay
Antioxidant test conducted on DPPH radical compound which has been recognized as an indicator of the antioxidant test. Search series test concentrations do some treatment and found a series of test concentrations. DPPH concentration used was 30 ppm, and an UV-VIS spectrometer. Concentrations of test series found are shown in Table 2.

Based on the concentration of the test series can be ascertained that the leaf extract antioxidant IC50 value kolowe including a very strong category which is <50 ppm.

c. Antimicrobial assay
Antimicrobial Test is still a screening against 19 types of microbes of which is a fungus. Microbes used are shown in Table 3.

d. Cytotoxic and antioxidant stability assay.
Cytotoxic and antioxidant activity extracts stability testing by storage time. Storage extract performed on the refrigerator with brown bottled container. Storage in an open space or room temperature is not done to avoid fungal invasion. The design of the stability testing of the two activities is shown in Table 4.
RESULT AND DISCUSSION
Secondary Metabolites profiles with Chemical Methods
Class of secondary metabolites was identified in methanol crude extract from Kolowe leaves are flavonoids, phenolics, saponins, steroids, and triterpenes. Triterpenes and steroid saponin aglycone which allegedly is becoming artifacts, such as flavonoids and phenolic tannins, the dominant content of secondary metabolites from Kolowe leaf are saponins, flavonoids, and tannins possibility as polyphenols. The interesting thing is quantitatively the content of secondary metabolites ethyl acetate extract fraction showed highest with 55.2% of the crude extract, 32.88% methanol fraction of the crude extract; whereas the n-hexane fraction lowest at 0.76% of the crude extract methanol. Due n-hexane fraction did not proceed at this stage of the tests. The results of the screening of secondary metabolites by chemical methods are shown in Table 5. Triterpenes and steroids detected an aglycone triterpene saponins and steroidal saponins and are not a triterpene and steroid free. Triterpenes and steroid allegations free artifacts from saponin. Because the results of the analysis by LC-MS / MS is not found free molecular profile triterpenes and saponins.

Chemical Profile Based on analysis of LC-MS / MS
Analyzes compound content profile from Kolowe leaf with LC-MS / MS made to extract fraction methanol and ethyl acetate fractions. Results of chemical screening earlier indicated that secondary metabolites are predominantly saponins and flavonoids. The chemical screening was conducted on crude extracts that contain secondary metabolites fraction of methanol and ethyl acetate can not be predicted. Analysis of LC-MS / MS against both factions will reveal the molecular mass compounds based on profiles and predictive analysis of compounds. Profile of secondary metabolites are predictive for LC-MS / MS which only determines the molecular weight compounds as well as fragmentation, but can not reveal the characteristics of every atom, cluster, and bonding the corresponding chemical environment in a compound. Profile compound believed to be a prediction based on the degree of similarity as well as a model compound structure prevalent if the same molecular mass with the results of the analysis.

a. Secondary Metabolites Profile compounds Methanol Fraction (EM)
Methanol extract fraction extract obtained from the solid-liquid fractionation using silica gel as the solid phase and methanol as mobile phase (liquid). Extract the methanol fraction was then analyzed by LC-MS/MS to determine the compound's profile abortion. Secondary metabolite profiles in the methanol fraction shown in Table 6. The analysis results illustrate that the compounds in extracts of methanol fraction are saponin glycosides and flavonoids, which consists of 10 species and 10 types of flavonoids saponin glycosides. Here the spectrum of LC-MS/MS EM compound 01-EM to 20-EM.

b. Secondary Metabolites Profile compounds Ethyl acetate Fraction (EA)
Ethyl acetate extract fraction extract obtained from the solid-liquid fractionation using silica gel as the solid phase and ethyl acetate as the mobile phase (liquid). Extract the methanol fraction was then analyzed by LC-MS / MS to determine the compound's profile abortion. Secondary metabolite profiles in the methanol fraction shown in Table 7 and Figure 3. The analysis results illustrate that the compounds in the extract fraction of ethyl acetate are saponins and flavonoids glycosides, which consists of five saponins and 3 compounds flavonoid glycosides.

Cytotoxic potential
Criteria cytotoxic materials have toxicity with LC_{50} values of <30 ppm, although the use of bio-indicators of the test using the larvae of A. salina [1]. The test cytotoxic results of Kolowe leaf extract (C. excelsus) shown in Table 8. The results of these tests show that the crude extract of methanol, the methanol extract fraction, and ethyl acetate fraction potentially cytotoxic-based criteria. The cytotoxic activity fairly stable at room temperature or in the refrigerator with the storage time of up to 16 weeks. The results of a third cytotoxic stability test of the extract are shown in Table 9.

Antioxidant Potential
Antioxidants could potentially have strong criteria which is 50 > LC_{50} < 100 ppm and strongest activity 0 > LC_{50} < 50 ppm [1]. Kolowe seed extract comprising methanol crude extract, extract a fraction of methanol, and ethyl acetate fractions including a very powerful antioxidant based on test results with DPPH radical compounds. The test results of the three antioxidant extract are shown in Table 10. Antioxidant extracts of the Kolowe leaves quite stable at room temperature which is safe in the refrigerator for a long 16 weeks or four months. During storage changes, IC_{50} values are meaningless and still in the powerful category of antioxidant. The results of stability test Kolowe leaf extract antioxidant shown in Table 11.

Antimicrobial activity
Antimicrobial Test of screening against 19 types of microbes comprising gram-positive bacteria, gram-negative, and mushrooms. All microbes reacted positively or extract can kill or inhibit the growth of microbes. This is a potential that can be followed up by research on the measurement of the inhibitory zone. The test results are shown in Table 12.

Tabel 1. Concentration series of Kolowe leaf extract (C. excelsus) for cytotoxic assay against A. salina larvae.

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>CONCENTRATION SERIES (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>Methanol crude extract</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate Fraction extract</td>
<td></td>
</tr>
<tr>
<td>Methanol Fraction extract</td>
<td></td>
</tr>
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</table>

113
Table 2. Concentration series of Kolowe leaf extract (C. excelsus) for antioxidant assay against DPPH

<table>
<thead>
<tr>
<th>EXTRACT</th>
<th>CONCENTRATION SERIES (ppm)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
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<td>5</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Methanol Fraction extract</td>
<td></td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>12</td>
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<tr>
<td>Ethyl acetate Fraction extract</td>
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<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
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</table>

Table 3. Types of microbe for antimicrobial activities from kolowe leaf extract (C. excelsus)

<table>
<thead>
<tr>
<th>NO</th>
<th>MICROBE</th>
<th>INFORMATION</th>
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<tr>
<td>1</td>
<td>Salmonella thyphosa,</td>
<td>Bacteria</td>
</tr>
<tr>
<td>2</td>
<td>Clostridium tetani,</td>
<td>Bacteria</td>
</tr>
<tr>
<td>3</td>
<td>Pneumonia carinii</td>
<td>Fungi</td>
</tr>
<tr>
<td>4</td>
<td>Candida albican</td>
<td>Fungi</td>
</tr>
<tr>
<td>5</td>
<td>Neisseria gonorrhoeae</td>
<td>Bacteria</td>
</tr>
<tr>
<td>6</td>
<td>Treponema pallidum</td>
<td>Bacteria</td>
</tr>
<tr>
<td>7</td>
<td>Coxiella burnetii</td>
<td>Bacteria</td>
</tr>
<tr>
<td>8</td>
<td>Clostridium tetani</td>
<td>Bacteria</td>
</tr>
<tr>
<td>9</td>
<td>Clostridium botulinum</td>
<td>Bacteria</td>
</tr>
<tr>
<td>10</td>
<td>Bacillus anthracis</td>
<td>Bacteria</td>
</tr>
<tr>
<td>11</td>
<td>Propionibacterium acnes</td>
<td>Bacteria</td>
</tr>
<tr>
<td>12</td>
<td>Vibrio paraahemolyticus</td>
<td>Bacteria</td>
</tr>
<tr>
<td>13</td>
<td>Streptococcus mutans</td>
<td>Bacteria</td>
</tr>
<tr>
<td>14</td>
<td>Agrobacterium tumefaciens</td>
<td>Bacteria</td>
</tr>
<tr>
<td>15</td>
<td>Streptococcus pneumonia</td>
<td>Bacteria</td>
</tr>
<tr>
<td>16</td>
<td>Pseudomonas solanacereum</td>
<td>Bacteria</td>
</tr>
<tr>
<td>17</td>
<td>Chlamydia trachomatis</td>
<td>Bacteria</td>
</tr>
<tr>
<td>18</td>
<td>Treponema pallidum pertenue</td>
<td>Bacteria</td>
</tr>
<tr>
<td>19</td>
<td>Staphylococcus aureus</td>
<td>Bacteria</td>
</tr>
</tbody>
</table>

Table 4. Design of stability studies cytotoxic treatment and antioxidants Kolowe leaf extract (C. excelsus)

<table>
<thead>
<tr>
<th>Storage time</th>
<th>Date and Assay replication</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st – 27th January 2016</td>
<td>28th – 31th January 2016</td>
<td>4 times assay with 12 replication</td>
</tr>
<tr>
<td>1st – 27th February 2016</td>
<td>28th Feb. – 2 March 2016</td>
<td></td>
</tr>
<tr>
<td>3rd s/d 29th March 2016</td>
<td>30th March – 2 April 2016</td>
<td></td>
</tr>
<tr>
<td>3rd s/d 29th April 2016</td>
<td>Tanggal 30 April – 3 Mei</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Secondary metabolite group from Kolowe leaf result (C. excelsus) with chemical method

<table>
<thead>
<tr>
<th>No</th>
<th>Secondary Metabolite group</th>
<th>Result</th>
<th>information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Triterpene</td>
<td>+++</td>
<td>Dominant</td>
</tr>
<tr>
<td>3</td>
<td>Steroid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Saponin</td>
<td>+++</td>
<td>Dominant</td>
</tr>
<tr>
<td>5</td>
<td>Flavanoid</td>
<td>++</td>
<td>Dominant</td>
</tr>
<tr>
<td>6</td>
<td>Phenolic</td>
<td>++</td>
<td>Dominant</td>
</tr>
</tbody>
</table>

Table 6. Secondary metabolite profile methanol fraction extract from kolowe leaf (C. excelsus) with LC-MS/MS

<table>
<thead>
<tr>
<th>Code</th>
<th>Retention Time</th>
<th>(M + H+)m/z</th>
<th>Molecule Weight (MW)</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>01-EM</td>
<td>3,720</td>
<td>440</td>
<td>439</td>
<td>Triterpene or flavanoid glycoside</td>
</tr>
<tr>
<td>02-EM</td>
<td>3,431</td>
<td>455</td>
<td>454</td>
<td>Triterpene or flavanoid glycoside</td>
</tr>
<tr>
<td>03-EM</td>
<td>0,422</td>
<td>365</td>
<td>364</td>
<td>flavanoid glycoside</td>
</tr>
<tr>
<td>04-EM</td>
<td>0,343</td>
<td>362</td>
<td>361</td>
<td>flavanoid glycoside</td>
</tr>
<tr>
<td>05-EM</td>
<td>4,540</td>
<td>268</td>
<td>267</td>
<td>flavanoid glycoside</td>
</tr>
<tr>
<td>06-EM</td>
<td>4,365</td>
<td>440</td>
<td>439</td>
<td>Triterpene or flavanoid glycoside</td>
</tr>
<tr>
<td>07-EM</td>
<td>4,240</td>
<td>1063</td>
<td>1064</td>
<td>Saponin triterpene</td>
</tr>
<tr>
<td>08-EM</td>
<td>4,129</td>
<td>749</td>
<td>748</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>09-EM</td>
<td>3,985</td>
<td>343</td>
<td>342</td>
<td>flavanoid glycoside</td>
</tr>
<tr>
<td>10-EM</td>
<td>5,811</td>
<td>719</td>
<td>718</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>11-EM</td>
<td>5,646</td>
<td>639</td>
<td>638</td>
<td>Saponin steroid</td>
</tr>
<tr>
<td>12-EM</td>
<td>5,560</td>
<td>227</td>
<td>226</td>
<td>flavanoid</td>
</tr>
<tr>
<td>13-EM</td>
<td>4,987</td>
<td>406</td>
<td>405</td>
<td>flavanoid glycoside</td>
</tr>
<tr>
<td>14-EM</td>
<td>6,526</td>
<td>593</td>
<td>594</td>
<td>Saponin steroid</td>
</tr>
<tr>
<td>15-EM</td>
<td>6,379</td>
<td>651</td>
<td>850</td>
<td>Saponin triterpene</td>
</tr>
<tr>
<td>16-EM</td>
<td>6,151</td>
<td>637</td>
<td>636</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>17-EM</td>
<td>6,114</td>
<td>949</td>
<td>948</td>
<td>Saponin triterpene/sterol</td>
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<tr>
<td>18-EM</td>
<td>7,872</td>
<td>311</td>
<td>310</td>
<td>flavanoid glycoside</td>
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<tr>
<td>19-EM</td>
<td>7,814</td>
<td>719</td>
<td>718</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>20-EM</td>
<td>6,590</td>
<td>851</td>
<td>850</td>
<td>Saponin triterpene/sterol</td>
</tr>
</tbody>
</table>

Table 7. Secondary metabolite profile ethyl acetate fraction extract from kolowe leaf (C. excelsus) with LC-MS/MS

<table>
<thead>
<tr>
<th>Code</th>
<th>Retention Time</th>
<th>(M + H+)m/z</th>
<th>Molecule Weight (MW)</th>
<th>Prediction</th>
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<tbody>
<tr>
<td>01-EA</td>
<td>7,807</td>
<td>755</td>
<td>754</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>02-EA</td>
<td>6,440</td>
<td>625</td>
<td>624</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>03-EA</td>
<td>6,201</td>
<td>1483</td>
<td>1482</td>
<td>Saponin triterpene</td>
</tr>
<tr>
<td>04-EA</td>
<td>6,151</td>
<td>1384</td>
<td>1383</td>
<td>Saponin triterpene</td>
</tr>
<tr>
<td>05-EA</td>
<td>4,909</td>
<td>719</td>
<td>718</td>
<td>Saponin triterpene/sterol</td>
</tr>
<tr>
<td>06-EA</td>
<td>4,540</td>
<td>354</td>
<td>353</td>
<td>Flavanoid glycoside</td>
</tr>
<tr>
<td>07-EA</td>
<td>4,368</td>
<td>376</td>
<td>375</td>
<td>Flavanoid glycoside</td>
</tr>
<tr>
<td>08-EA</td>
<td>4,111</td>
<td>240</td>
<td>239</td>
<td>Flavanoid glycoside</td>
</tr>
</tbody>
</table>

Table 8. Cytotoxic assay result Kolowe leaf extract (C. excelsus) against A. salina larvae.

<table>
<thead>
<tr>
<th>No</th>
<th>Extract</th>
<th>LC50 (ppm)</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol crude extract</td>
<td>15,44</td>
<td>cytotoxic</td>
</tr>
<tr>
<td>2</td>
<td>Methanol Fraction extract</td>
<td>16,28</td>
<td>cytotoxic</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate Fraction extract</td>
<td>8,88</td>
<td>cytotoxic</td>
</tr>
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</table>
Figure 2. Spectrum profile of LC-MS/MS 01-EM – 20-EM compound from methanol fraction Kolowe leaf 

(C. excelsus)
Figure 3. Spectrum profile of LC-MS/MS compound 01-EA – 08-EA from ethyl acetate fraction extract of Kolowe leaf (*C. excelsus*).
Table 9. Cytotoxic stability assay result Kolowe leaf extract (C. excelsus) storage time.

<table>
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<th>No</th>
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<th>storage time (week)</th>
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<tbody>
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<td>1</td>
<td>Methanol crude extract</td>
<td>15.44</td>
<td>17.64</td>
<td>18.82</td>
<td>18.78</td>
<td>18.48</td>
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<td>Methanol Fraction extract</td>
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<td>18.42</td>
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<td>19.34</td>
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<td>Ethyl acetate Fraction extract</td>
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<td>11.14</td>
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Table 10. Antioxidant result from Kolowe leaf extract (C. excelsus) against DPPH

<table>
<thead>
<tr>
<th>No</th>
<th>Extract</th>
<th>IC50 (ppm)</th>
<th>Antioxidant activity</th>
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<td>1</td>
<td>Methanol crude extract</td>
<td>4.44</td>
<td>strong</td>
</tr>
<tr>
<td>2</td>
<td>Methanol Fraction extract</td>
<td>6.56</td>
<td>strong</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl acetate Fraction extract</td>
<td>3.34</td>
<td>strong</td>
</tr>
</tbody>
</table>

Table 11. Antioxidant stability test from Kolowe leaf (C. excelsus) storage time.

<table>
<thead>
<tr>
<th>No</th>
<th>Extract</th>
<th>IC50 (ppm)</th>
<th>storage time (week)</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methanol crude extract</td>
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<td>5.76</td>
<td>4.86</td>
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<tr>
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<td>Methanol Fraction extract</td>
<td>6.56</td>
<td>8.43</td>
<td>8.24</td>
<td>7.72</td>
<td>7.04</td>
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<tr>
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<td>Ethyl acetate Fraction extract</td>
<td>3.34</td>
<td>4.44</td>
<td>4.03</td>
<td>3.84</td>
<td>3.55</td>
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</tr>
</tbody>
</table>

**CONCLUSION**

1. Secondary metabolites Kolowe leaf based on chemical screening are saponins and flavonoids and steroids. Triterpenes and steroids detected an aglycone triterpene saponins and steroid saponins or saponin artifacts result.
2. Secondary metabolites profile by LC-MS / MS methanol and ethyl acetate extract fraction relatively the same and consists of saponin glycosides and flavonoids. This illustrates the fractionation technique is not optimal and founded classes of compounds that are same in both solvents.
3. Methanol crude extract, extract a fraction of methanol and ethyl acetate fraction Kolowe leaves potentially cytotoxic with average LC50 values <30 ppm; a powerful antioxidant category with an average IC50 of less than 50 ppm.
4. The crude extract of leaves Kolowe be antimicrobial against 19 types of microbes which are gram positive and negative bacteria and fungi.

**ACKNOWLEDGMENT**

Thanks to Head of the Laboratory of Biotechnology BPPT, Jakarta, which has helped in the analysis of LC-MS / MS.

**REFERENCES**