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Review Of Potential Saponins Extract From Kolowe Fruit Seed (*Chydenanthus excelsus*) As Pharmaceuticals : A Wild And Rare Plants from Indonesia

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Abstract

Kolowe (*Chydenanthus excelsus*) is a wild and rare plants as well as a small genus in the world, found only in the Andaman Islands, Myanmar and Buton islands, Indonesia. Seeds of this plant were found to contain saponins, spread in the fraction of n-butanol, methanol, methanol-water (7:3) and (1:1) to reach 41.96%. The saponins extract has several potential biological activity such as cytotoxic, antikoksidan, antimicrobials, and potentially toxic to humans.

Keywords : Saponin extract, Kolowe fruit seed, *Chydenanthus excelsus*

INTRODUCTION

Saponin is a naturally occurring compound mysterious because it has been known to have more than 21 kinds of biological activity associated with pharmaceutical potential [1]. Biological activity include antibacterial, antifungal, antiviral, cytotoxic, antimoluska, supermisida and contraceptives, insecticides and antimakan, anthelmintic, expectorant and antitussive, diuretic, lowering cholesterol (Cholesterol methabolism), the activity of Cardiovascular, antiinflammatory, antiexudative, antioedemotaus, antiulcer, analgesic , antipyretic, immunomodulation, adaptogenic, sedative, miscellaneous and others. Biological activity is a lot of potential, but required in-depth research on each class of steroidal saponins for example saponins, steroidal saponins alkaloids, saponins and alkaloids.

Utilization of saponin as a source of pharmaceutical materials difficult to be realized despite the potential biological activity of saponin which is very varied. Difficulty utilization of saponin compounds as pharmaceuticals due to the difficulty of separating the saponin compounds that should use the instruments of high-tech separation of chemical compounds, such as preparative HPLC column and so few that does not deserve to be exploited on a large scale. Businesses that can be done is the multiplication of the compound saponin extract or utilization of biotechnology as a source of pharmaceutical ingredients. Saponin extract is defined as an extract which only mengadung saponin compounds (a mixture of saponins) and do not contain other chemical compounds. Group saponins having very high polarity are generally extracted from the solvent n-butanol to the aqueous solvent, although some types of saponin especially groups steroidal saponins and alkaloids steroid saponins can be extracted in ethyl acetate solvent that is semipolar [4]. Problems to obtain a saponin extract using fractionation techniques of solid-liquid and liquid-liquid allowing a saponin extract were still terkontaminan with other chemical compounds as have similar physico-chemical properties of the compound saponin metabolites.

Interesting phenomenon of the class of compounds widely found biological saponin containing saponin and extracted as metabolites predominantly in the solvent n-butanol in the fractionation. Results of research saponin against 18 types of plants, namely *B. rigidum, Z. gactulum, D. surculosa, M. laxiflora, G. sinensis, B. chrysogonum, A. squarrosum, I. dumortieri, P. tuberosa, Myrsine spp, S. schomburgkii , M. japonica, P. patens, M. pubescens, C. foetidissima, T. palmata, L. hyalina,* and *Y. schidigera*; keseluruhannyan extracted in n-butanol in a fractionation system, except plants *L. hyalina* partially extracted in ethyl acetate [2]. The phenomenon may be a reference that contains saponins biological potentially obtain a saponin extract pure by using solvent n-butanol in the fractionation. Nevertheless tertenti needed a technique to obtain pure saponin extract of fraction of n-butanol, as many other secondary metabolites that can be extracted with n-butanol. The evidence suggests that general biodiversity mengndung dominant saponin as secondary metabolites and contain no more polar secondary metabolites. This occurs in fruit seeds that contain saponin kolowe pure mixture in n-butanol fraction [2]. The saponin fraction of a saponin extract pure mixture with 32.6% against the quantity of seed extract kolowe and not contaminated with other metabolites based on the analysis HPLP analytical column and isolated by preparative HPLC column. However, the fraction of water still contains saponin and has not performed chromatographic and spectroscopic identification, but only by chemical screening [2]. Kolowe (*C. excelsus*) Lecythidaceae is a family of wild plants and rare in the world and is a small genus [8]. This plant is only found in the Andaman Islands, Myanmar [8]. and in Kamaru Buton islands, Indonesia called kolowe [2]. This plant fruit throughout the year or not in season, although there are certain seasons fruitful very much [2]. Seeds of the plants used by the community Kamaru, Buton, Indonesia as marine fish poison which is used to catch fish by poisoning the fish in a sea area to a depth of 1.5 m. The toxicity of the seeds for this not to cause poisoning in humans who consume the fish with the seed. This illustrates that toxicology kolowe seed is not toxic to humans. Here's a picture Kolowe in Kamaru, Buton, Indonesia.

Based on these descriptions have been many studies conducted on grain yield determination kolowe include saponin extract and some biological activity. Determination

of pure saponin extract is essential for continued research purposes. Determining the yield of saponin extract from the seeds must be started from any purification saponin fractions containing saponin in order to obtain pure saponin extract. The technique is done is the addition of diethylether solvent in a solution containing saponins. The working principle is based on the expected high polarity difference between diethylether by dissolving the extract solution such as n-butanol, ethanol, methanol, water and others. Saponin extract will settle especially if the non-polar solvent is more dominant in solution that can be helped with a simple centrifugal. The purification techniques have been made to the saponin extracted from the seeds kolowe in n-butanol fraction. Ensuring the purity of saponin with these techniques were analyzed by HPLC analytical column and the saponin compounds isolated using preparative HPLC column as well as the molecular structure determined by spectroscopy (IR, ESIMS, 2D-NMR). The result is a whole grain kolowe metabolites in the n-butanol fraction are so-called saponin with saponin extract. Therefore, we conducted further research on seed kolowe committed to the fraction of methanol-water (7: 3), but has not done chromatographic ensuring high performance and also spectroscopically. Saponin extract obtained by purification techniques such saponin extract, then performed potential assay as pharmaceuticals saponin extract that is assay an antioxidant, cytotoxic, toxicity, and antimicrobial (bacteria and fungi).

MATERIALS AND METHOD

The results reported a review of the results from several studies of Kolowe seed *(C. excelsus)* undertaken since 2004. The results reported extract saponins from kolowe fruit seed focus on, compound and the amount of saponins extracts content of kolowe fruit seed, the best techniques of extraction saponins pure mixture of kolowe seeds, saponins extract antimicrobial activity of kolowe seed, cytotoxic and antioxidants and stability of the saponins extract kolowe seed and, *in vivo* toxicity saponins extract from kolowe seed . The research method described general nature of the research.

1. **The Extract yield and Compound type of saponin extract from Kolowe seeds**

   The purpose of these studies is to find a saponin extract pure mixture of seeds kolowe, determine the yield of saponins extract, isolate and determine the molecular structure of saponins from saponin extract. saponins extract is an extract containing only classes do not contain saponins and other metabolites. Determination of an extract as saponins extract with chemical screening techniques in the extract, saponins extract chromatogram pattern analysis by analytical HPLC, isolate and determine the structure of saponins from the extract. It is conducted on kolowe fruit seeds .

A **B** **C**

D **E** **F**

Figure 1. Kolowe (*C. excelsus*): A = Fruits and shoot; B = group of trees/forest ; C = flower; D = fruits; E = fruits harvesting; F = seeds and fruit skin (Rijai, 2004)

a. Determination of Saponin Extract from Kolowe Seed and Yield.

Saponin screening from the seeds of fresh fruit kolowe by chemical methods. The screening purposes to ensure that the seeds kolowe contain saponin . Extraction entire secondary metabolites from the seeds of fresh fruit kolowe with cold maceration method using methanol 96%. The extract was fractionated by liquid-liquid technique using a solvent gradient n-hexane-water, ethyl acetate-water, and the last n-butanol-water. Chemical screening including the saponin extract n-hexane, ethyl acetate, n-butanol and water fractions. Purification of saponin was done by using the addition of solvent diethylether at a certain amount on a saponin extract solution.

Addition of diethylether solvent to form a precipitate which allegedly is a saponin extract. These deposits are separated by the solvent and obtained an extract of a suspected saponin extract. Saponin extract the results of the screening deposition of secondary metabolites, including saponins. Screening results showed that the sediment is saponin, whereas negative saponin extract filtrat solution and also other negative secondary metabolites. Thus obtained kolowe seed saponin extract of fraction of n-butanol. Fraction positive water chemical saponin, but saponin extract purification has not been done. Determining the yield of seed saponin extract kolowe based solely on the amount of saponin extracted with a solvent n-butanol, while the saponin extract in a fraction of the water purification has not been done. The entire deposition saponin from n-butanol fractions weighed to determine the yield of the saponin extract from kolowe seed.

b. Saponin compound isolation from Kolowe fruit seeds.

Saponins compound in saponin extract (fraction of n-butanol) kolowe seed need isolation procedure to obtain pure compounds. Isolation technique of saponins compound were performed using preparative HPLC column which was preceded by a qualitative analysis of saponin compounds using HPLC analytical column. HPLC system analytical column used was Waters 510 pump; Waters U6K injector; Waters 490E UV detector with wavelength 254 nm and Waters R410 RI; Alltech column Alltima C18 - 86 reversed phase, with column size 25 cm x 4.8 mm and a particle size of 5 lm. Eluent used was methanol-water-acetic acid (75: 25: 0.5). Furthermore, the saponins which are identified from analytical HPLC column were isolated using preparative HPLC column. HPLC systems preparative columns used are Waters 510 EF pump; injector Rheodyne 7125; Waters 841 UV detector wavelength 250 nm and Waters R403 RI. Eluent used was methanol-water-acetic acid (75: 25: 0.5 ml) with a flow rate of 11,25mL / min uses Alltima Alltech column C18 reverse phase column size 30 x 2.2 cm with a particle size of 10 μm. The results of analytical HPLC analytical column against a saponin extract (fraction of n-butanol) seeds kolowe generated 25 peak that illustrates there are 25 kinds of saponins, while the isolated compound with HPLC systems preparative columns found three types of compound and two compounds of which came from one crystalline amorphous.

c. The structure elucidation of saponins from Kolowe seeds

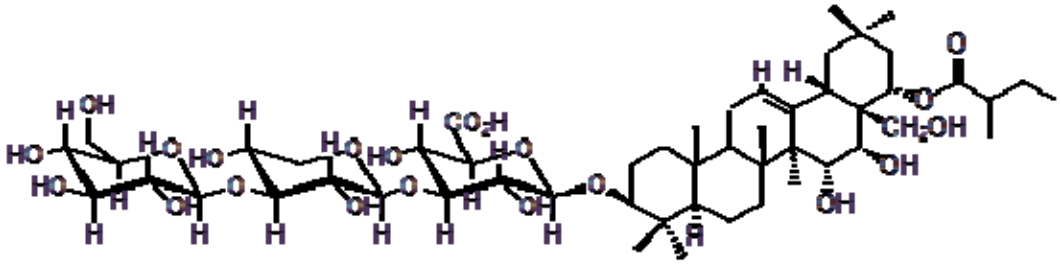
Kolowe fruit seeds have been found three compounds that are triterpene saponins in 2004. Structure elucidation technique of three compounds using FTIR spectroscopy; 2D-NMR and EIMS. The results of the analysis of HPLC analytical column was found 25 peak in the chromatogram, but which can be determined only three compound structure. One of the amorphous crystal of 2 crystalline amorphous discoverability and analytical HPLC showed one sharp peak, but spectroscopically-2D NMR 400 MHz turns the amorphous crystalline compound containing 2 berisomer triterpene saponins. Furthermore, recording the IR spectrum used by nujol techniques from Shimadzu FTIR 8400S, whereas MS analysis using electrospray technique Ionization Mass Spectrometry (EIMS). MS spectrometer used is Finningan MAT900 XL chromatography for separation LCQMS. Further NMR used was a Bruker Avance 400 frequency 400 MHz for 1H NMR and 100 MHz for 13C NMR. The techniques used are HH-COSY; ROESY-2D; ROE-D; DEPT; HMQC; HSQC; TOCSY-1D; and HMBC.

2. Optimization of Saponin Extraction Technique from Kolowe Seed

Interest optimizing extraction technique of saponins kolowe seed to find the best extraction solvent that produces pure saponin extract at most compared to other solvents. This study draws on research saponins previous isolation from the seed that is in addition to saponins found in the n-butanol fraction of the liquid-liquid fractionation techniques, there is still a fraction of saponin in water. The research method is the use of a solvent used is methanol 96%; methanol-water (7: 3) and methanol-water (1: 1). On the third such solvents do macerated fresh fruit seed powder kolowe until the whole extract of the seeds out extracted with these solvents. Each of the extract was fractionated by using solid-liquid gradient using a vacuum pump as a towing extract solution with a stationary phase silica gel GF60 with n-hexane, ethyl acetate, n-butanol, methanol 96%, methanol-water (7: 3) and methanol-water (1: 1). The whole extract fractions obtained qualitative screening techniques saponin dilution with distilled water. Extract fractions were shown to contain saponin fractions is extract n-butanol, methanol and 96% methanol-ai (7: 3) and methanol-water (1: 1). Saponin

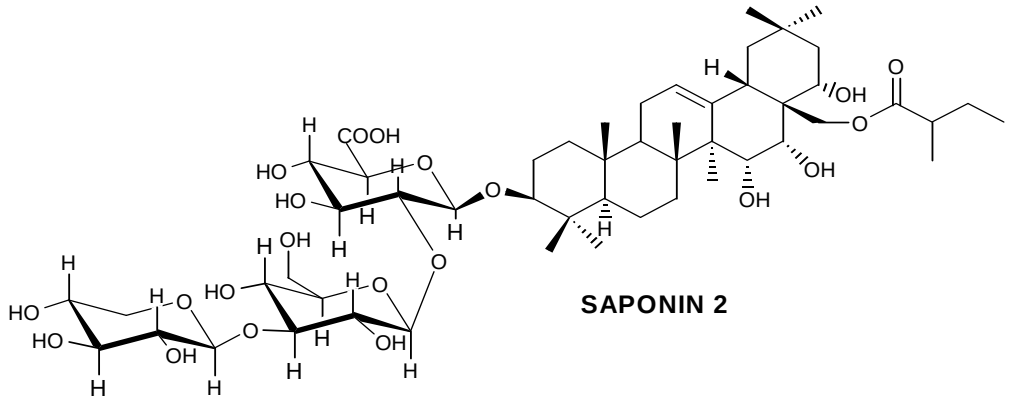
extract the extract solution fractions were added with diethylether solvent so that a precipitate is formed with the help of a simple centrifugal deposition is a saponin extract. Saponin extract in the form of the sediment screening saponins and other secondary metabolites, as well as over a saponin extract filtrate. The entire saponin extract from each fraction weighing is done analytically to determine the weight of pure saponin extract in each fraction. Saponin extract from a certain faction with much more than the other factions, defined as the best solvent for extracting saponin from kolowe seeds. The solvent extraction technique is a new finding saponin from kolowe seeds.

3. **Antimicrobial Assay Pure saponin Extract from Kolowe Seed**
   Objective of this study to determine the potential saponins extract kolowe seed as an antibacterial, especially against some bacteria and fungi. Microbes special test in which harm humans directly or indirectly. Tests are only screening which is both active and inactive. Which microbes were subjected to the test are shown in Table 2.
4. **Cytotoxic and Antioxidant Assay of saponin Extract from Kolowe Seed**
   Cytotoxic and antioxidant Assay done simultaneously against saponins extract kolowe seed in the hope of potential for both of these events. Both tests which is performed *in vitro* cytotoxic against larvae of *A. salina*, while trials of antioxidants on DPPH free radical compounds. Tests to the determination of the LC_{50} value for cytotoxic and IC_{50} test for the antioxidant test. In addition, test the stability of the cytotoxic and antioxidant treatment with storage time of up to 16 weeks.
5. **Toxicity assay of saponin Extract from Kolowe Seed**
   The test results cytotoxic and antioxidant of saponin extract from kolowe seed shows great potential as a cancer drug since both having an LC_{50} and IC_{50} <30 ppm. However, toxicity to *A. salina* <30 ppm, also categorized as toxic, potentially cytotoxic. However, a natural compound has mysterious properties including saponins. Because it needs a simple toxicity testing in vivo with test parameters based on changes in SGOT and SGPT blood of experimental animals. Step tests were conducted experimental design comprised the control group, 1-dose group (equal to the LC_{50}) and the 2-dose group (2 times the dose-1) with three replications. Treatment kolowe seed saponin extract solution with a dose equal to the value at the test LC_{50} cytotoxic (dose-1) and 2 times the dose of the LC_{50} value (dose-2). Giving oral extract solution according to the dosage for 6 consecutive days at a stretch, and on the seventh day measured levels of SGOT and SGPT blood test animals. The problem of this study is the homogeneity of the test animals can not be achieved including the animal's body weight.



SAPONIN 1

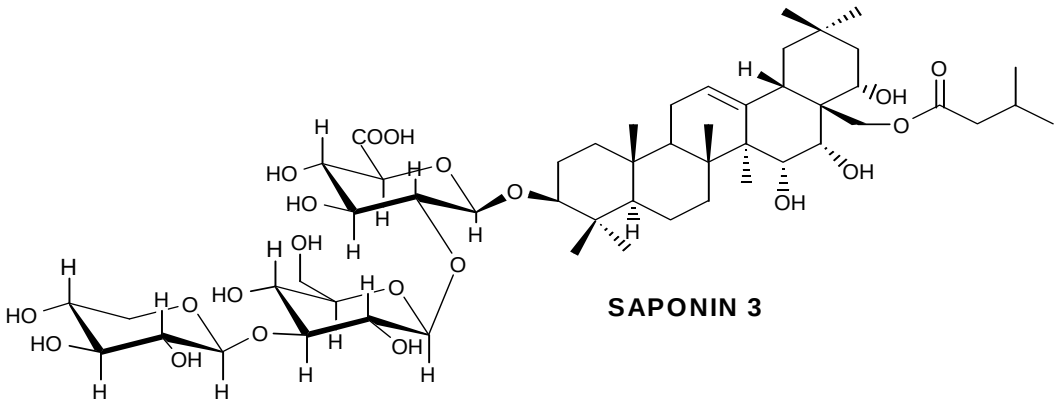
3-*O*-[β-D-glucopyranocyl (1→3)- β-D-xylopyranocyl(1→3)- β-D-glucuronopyranocyloxyl] -22α-*O*-(2-methylbuthyroyloxyl)-oleana-12-en-15α,16α, 28-trihydroxy.



SAPONIN 2

3-*O*-[β-D-Xylopyranocyl (1→3)-β-D-glucopyranocyl (1→2)-β-D-glucuronopyranocyloxy]-28-*O*-(2-methylbuthyroyloxy)-oleana-12-en-15α, 16α, 22α-trihydroxy.

Figure 2. Saponin from *n*-butanol fraction of kolowe seeds (Rijai, *et al*., 2004)



3-*O*-[β-D-Xylopyranocyl (1→3)-β-D-glucopyranocyl (1→2)-β-D-glucuronopyranocyloxy]-28-*O*-(3-methylbuthyroyloxy)-oleana-12-en-15α, 16α, 22α-trihydroxy.

Figure 3. Saponin from *n*-butanol fraction of kolowe seeds (Rijai, *et al*., 2004)

RESULT AND DISCUSSION

1. Extract and Saponins Compounds of kolowe seeds

Saponins extract is an extract containing saponins group and contains no other metabolites, while saponins, a compound saponins known molecular structure. Kolowe Seeds (*C. excelsus*) a rare wild plants and is the least in the world genus proved to contain a saponin extract and compound of saponin.

a. Saponin extract from the Kolowe seeds.

Saponins extract from kolowe seeds in liquid-liquid fractionation system that is n-hexane-water; ethylacetate-water; and n-butanol-water, spread on a fraction of n-butanol and water fractions. Saponins extract were dispersed in n-butanol fraction with the fractionation system as much as 32.6% of the total seed extract kolowe, while saponins are dispersed in water undetermined fraction of weight. This indicates that the kolowe seed containing saponins extract > 32.6%, due saponins fractions scattered on the water with such a system has not been analyzed. The weight of saponins extract a high enough that a potential, due to the utilization of saponins in compound form is difficult to conducted, but only for the marker compound. Potential saponins in the pharmaceutical field is quite a lot that has more than 21 kinds of biological activity. Utilization in the form of compounds in the pharmaceutical field is difficult to conducted because of the weight of saponins compounds that are often found very low and also have difficulty in separation. Thus the content of saponins extract in kolowe seeds that reach> 32.6% is a great potential as a source saponins.

b. Saponin compoundt from the Kolowe seeds.

Saponin extract from the kolowe seed of n-butanol fraction had to be identified by analytical HPLC chromatography and generate 25 peak in the chromatogram. This illustrates that the saponin extract of n-butanol fraction kolowe seeds contains at least 25 kinds of saponins. The results of analytical HPLC analysis of the extracts of saponin also supports the results of the saponin extract chemical screening, namely that the n-butanol fraction of a saponin extract because it contains no other metabolites. Corak saponin extract analytical HPLC chromatogram of n-butanol fraction terseut kolowe seeds, also shows a mode spectrum of analytical HPLC prevalent in saponin extract. Ascertainment of n-butanol fraction kolowe seeds as a saponin extract followed by isolating and mngelusidasi saponin structure of the faction. Isolation saponins which uses techniques preparative HPLC column was found two amorphous crystal which is a compound saponin. The results of spectroscopic analysis (FTIR, EIMS, 1H NMR and ^{13}C NMR 400 MHz to 100 MHz) of two amorphous crystals were found three compounds that the two compounds are from 1 crystalline amorphous which is a saponin berisomer. This incident shows that HPLC analytical column still has a weakness for separating compounds berisomer saponin. Saponins indeed have similar physico-chemical properties are quite high that it is difficult to be separated. Similarities physico-chemical properties will be higher in saponin berisomer compound with a structure that is almost the same. The findings of the three types of compounds from seed saponin extract kolowe reinforce the data that n-butanol fraction kolowe seed is a saponin extract is an extract containing only compound saponin class. Saponins are recovered saponin extract from kolowe seed shown in Figures 2 and 3.

c. Yield and sponin extraction technique from kolowe seed

Percentage of Saponin extract from kolowe seeds described earlier, has not the yield because the saponins extract derived from n-butanol fraction in the liquid-liquid fractionation system, while saponins from kolowe seeds scattered on a fraction of n-butanol and water used in the fractionation system. Because the percentage of saponin extract specified> 32.6%. In this section we report Saponin extract from kolowe seeds yield more precise than before although it has limitations. Also found the best solvent for extracting saponins from kolowe seeds is a solvent extraction that produces a saponin extract that much. Kolowe seed saponins extraction with various solvents ratio shown in Table 1.

Table 1. Saponins extract weights of 410g of crude extract seed kolowe (C. excelsus) using multiple comparisons solvent extraction with cold maceration method and solid-liquid fractionation

No	Solvent	Weight of saponin Extract Kolowe Seed on Solvent Fractionation by Solid-Liquid Technique (g)				Yield of Saponin extract (%)
		n-Butanol	Methanol 96%	Methanol-water (7 :3)	Methanol-water (1 : 1)	
1	Methanol 96 %	86,48	20,06	8,44	4,46	29,13
2	Methanol-water (7:3)	122,64	28,66	12,26	8,40	41,94
3	Methanol-water (1:1)	82,82	12,46	4,68	2,44	24,97

Sources: Rijai et al., 2012

Table 1 illustrates that the best solvent for extracting saponins from fruit seeds kolowe are methanol-water with a ratio of 7: 3. In addition, the polarity of the compound saponin kolowe seeds varies greatly, ranging from n-butanol to methanol-water (1: 1). This supports previous research that saponin kolowe seeds are triterpene saponins and these groups are very polar saponins. Extracts found in fractions of 1.2 g of n-hexane and ethylacetate fraction of 76.43 g. It also shows there is a saponin still allow the fraction of water that was not done in this study fractionation. Thus the best yield of extraction solvent methanol-water (7: 3) in Table 1 are still relative is supposed to be> 41.94%. At the ethyl acetate fraction contained no saponin based chemical screening, however chemical screening can only be detected in sufficient amount of a compound of this class of compounds were examined with reagent (solvent water to saponins).

Based on these reports, the fruit seeds kolowe potential as a source saponins, while saponins are a class of natural compounds that have more than 21 kinds of biological activity. Saponin extract can also be used for blood hemolysis and various other marker compounds. The economic potential of saponins very prospect that further research on plants kolowe indispensable.

2. Antimicrobial activity of saponin extract from the kolowe seed

Potential kolowe seed as a potential source of saponins become motivation to continue research on seed kolowe. The studies were conducted among others as an antimicrobial with representatives of parasitic bacteria, parasitic bacteria pathogenic and pathogenic bacteria but only limited to the *E. coli, S. aureus, S. pseudomonas* bacteria as well as antifungal *C. albicans.* Selection of types of microbes are not yet under consideration for development, but it is still an activity screening kolowe seed saponins extract as an antimicrobial. However, tests performed up to the measurement of inhibitory zone or manually kill zone. Antimicrobial test results are shown in Table 2.

Table 2 shows that screening for 19 types of microbial bacteria and fungi, all provide positive activities. In chemistry the subject is appropriate, since the end of the discussion is saponin compounds are polar and non-polar which will cause tension in the bond when interacting with non-polar or polar components such as walls interrupted microbes.

Table 2. Results antimicrobial assay of saponins extract from kolowe seed (C. excelsus)

NO	Microbe	Result (+)/(-)
1	*Salmonella thyphosa,*	++
2	*Clostrididum tetani,*	++
3	*Pneumonia carinii*	++
4	*Candida albycan*	+++
5	*Neisseria gonorrchoeae*	+++
6	*Treponema pallidum*	+
7	*Coxiella burnetii*	++
8	*Clostridium tetani*	++
9	*Clostridium botulinum*	++
10	*Bacillus anthracis*	+++
11	*Propionibacterium acnes*	+
12	*Vibrio parahaemolyticus*	+
13	*Streptococcus mutans*	+
14	*Agrobacterium tumefaciens*	++
15	*Streptococcus pneumonia*	++
16	*Pseudomonas solanacrearum*	++
17	*Chlamydia trachomatis*	++
18	*Treponema pallidum pertenue*	++
19	*Staphylococcus aureus*	++

Sources: Rijai, 2013

3. Cytotoxic and Antioxidant activity of saponin Extract from Kolowe seed.

Cytotoxic and antioxidants are a couple of potential biological activity associated with cancer drug material. Cytotoxic activity of killer cells act as abnormal or cancerous cells, while antioxidants stimulate regeneration of damaged cells. Kolowe seed saponins extract having both the *in vitro* activity qualifies as cytotoxic and antioxidant ingredients for the LC_{50} values against larvae of *A. salina* and IC_{50} against DPPH radical compounds respectively <30 ppm. In addition, the two activities are very stable at room temperature storage for 16 weeks. Antioxidant, cytotoxic assay and stability result are shown in Tables 3 and 4.

Table 3. The stability of the cytotoxic activity of saponin extract from kolowe fresh fruit seed with bioindikator larvae of A. salina and storage time of 16 weeks

No	Storage time (weeks)	LC_{50} (ppm)
1	0	14.88
2	4	16,25
3	8	16,65
4	12	16,60
4	16	16,45

Sources; ***Rijai et al., 2014***

Table 4. The stability of the antioxidant activity of saponin extract from kolowe fresh fruit seed with test indicators compound DPPH radical and storage time of 16 weeks

No	Storage time (week)	IC_{50} (ppm)
1	0	22,45
2	4	25,80
3	8	26,22
4	12	26,42
4	16	26,42

Sources; Rijai, 2014

4. Toxicity of Saponin extract from Kolowe seeds

Potential cytotoxic *in vitro* against *A. salina* larvae by the saponin extract from kolowe seeds also emphasizing that the extract have toxic effect *in vitro* with a value LC_{50} <30 ppm. Nevertheless, the events of the toxic compound is more systemic *in vivo* that these compounds can undergo molecular structure changes in the metabolic system and then become toxic or incurred directly by the intact molecule of the compound. The metabolism of living beings involves various enzymatic mechanisms, a variety of chemical signals as a hormone even genetic information in the system for the metabolism of nucleic acids and other metabolites that can act as receptor molecules against xenobiotics. Thus a toxic substance directly on a specific bio-indicators are not always toxic to other creatures in metabolism. Therefore the seed saponin extract kolowe toxicity test was also conducted *in vivo*, but only limited to the observation of changes in the levels of SGPT and SGOT test animal blood as an indicator of disruption in living beings, including humans.

The results of toxicity assay of saponins extract kolowe seed by changing levels of SGOT and SGPT test animals are shown in Tables 5 and 6. Criteria for determining toxic levels of a substance based on changes in AST and ALT is the change of SGOT and SGPT normal to 20 times or more than normal. Experiments conducted on kolowe seed saponins extract that is used in accordance with the test dose LC_{50} toxicity test results *in vitro* against A. salina, and also given the extreme dose is twice the dose of the LC_{50} value. The result, as Table 5 and 6 show that changes in the levels of SGOT and SGPT not menacapai 20 times the levels of SGOT and SGPT test animals without extract treatment so that it can be stated that the seed saponins extract kolowe potentially toxic. It is a portrait that metabolic processes including the immune contribute significantly xenobiotic saponins extract from kolowe seed.

CONCLUSION

The best technique saponins extracted from the seeds of fresh Kolowe fruits (*C. excelsus*) using methanol-water with a ratio of 7: 3 to yield reaches up to 41, 94%. Kolowe seeds have potential as a source of saponin extract because the yield is > 41.94% in the use of solvent extraction with methanol-water (7: 3). Saponins from Kolowe fruit seeds highly polar, which is extracted in the solvent n-butanol, methanol, methanol-water ratio of 7: 3 and 1: 1; and a compound triterpene saponins mainly found in the fraction of n-butanol. Potential saponins extract from the seeds of kolowe fresh fruit as a source of pharmaceutical materials which have a cytotoxic activity, antioxidants, antimicrobials, as well as potentially not toxic to humans.

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Table 5. Result of in vivo toxicity assay saponins extract from kolowe fresh fruit seed (C. excelsus) with bioindicator white rat and treated for 5 weeks based on the change of SGOT

Saponin extract Dose	Average levels of AST (IU) measurement Period (weeks)		
	I	III	V
Without saponin extract	44,2	49,4	46,8
3,3, mg (same with LC_{50} extract value)	107,2	112,8	72,3
6,6 mg (2 times LC_{50} extract value)	144,5	134,5	67,4

Sources: Rijai., 2015

Table 6. Results of in vivo toxicity test saponins extract Kolowe fresh fruit seed (C. excelsus) with bioindicator white rat and treated for 5 weeks based on the change in the levels of alanine aminotransferase

Saponin extract Dose	Average levels of AST (IU) measurement Period (weeks)		
	I	III	V
Without saponin extract	24,6	22,4	20,6
3,3, mg (same with LC_{50} extract value)	48,2	30,4	21,6
6,6 mg (2 times LC_{50} extract value)	82,1	110,6	42,4

Sources: Rijai., 2015

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