

# Review of Fast Technics for Isolation and Structure Elucidation of Triterpene Saponins Compound from Bionatural Products

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## Abstract

Saponins have specific characteristics are highly polar, generally, as a compound isomers, diversity of this compounds founded with a large number in living natural material, complex chemical structure and physicochemical properties of the compound is very similar to one another. Characteristics such as a major cause difficulty separation of the compound and the difficulty in determining the molecular structure by conventional techniques. Triterpene saponins for the separation more difficult because more polar, as well as the elucidation of the structure because the structure is more complex. Isolation technique of saponins most rapidly and precisely locate compound saponin saponin extraction using methanol-water (7: 3 or 1: 1), the purification of saponin mixture with the addition of ether solvent in a solution containing saponin fractions, isolation techniques by preparative HPLC or semi- preparative reversed-phase HPLC eluent with the determination of the analytical column; and for the determination of the structure quickly and accurately using  $^1\text{H}$  and  $^{13}\text{C}$  NMR-2D frequency  $\geq 400$  MHz techniques DEPT, HH-COSY, HMBC, HMQC / HSQC, ROESY-2D, ROE-1D, TOCSY-1D, and spectrometry FTIR and FABMS like ESIMS.

**Keyword:** Isolation technique, Structure elucidation, saponin triterpenoid.

## INTRODUCTION

Research of saponin from natural materials is very potential in science and economics. In science, every research saponin likely finds new compounds, discover new potentials mainly related to pharmacy, and generate knowledge and experience skills to implement separation of natural compounds and determining the molecular structure of the compound. Furthermore, economically saponin extract and saponin compounds have the potential pharmacy larger because it has biological activity that many, physicochemical properties of specific and generally very dominant in a biological or one organ bio-certain to reach the yield  $> 46\%$  (Rijai, 2016). Utilization of saponin in the pharmaceutical field can be done in the form of saponin extract whose numbers are often very much on a biological or a biological organ. Therefore, research saponin from natural materials to be very interesting and potentially up to Indonesia has abundant biodiversity as sources of saponins. Generally, saponin is composed of two groups: steroidal saponins and triterpene saponins. Steroid saponins have the basic framework of the main cholestane, spirostan, furostan steroidal alkaloids and saponins with basic structure solanidine and spirosolans. Steroid saponin is less widespread in nature than the saponin triterpene (Hostettmann and Marston, 1995). Dominant steroidal saponins found in nature are spirostanol and furostanol. Spirostanol steroidal saponins are generally part of sugar bound to the atom C-3 while steroid furostanol saponin part sugar bound to the atom C-26. Monocotyledons plants are a major source of steroid saponins (Hostettman dan Marston, 1995). Spirostanol saponins have very many diversity of has nine core structure which may be substituted sapogenin sugar, hydroxyl and other substituents with different stereochemistry. In the atom C-25 saponin spirostanol

always contains substituents with R or S stereochemistry, while the atom C-1; C-2; C-3; C-4; C-5; C-6; C-12; C-14; C-15; C-17; C-21, C-24, generally bound in the form of  $\alpha$  or  $\beta$  position (Hostettmann and Marston, 1995). That situation illustrates that the diversity of steroidal saponins huge with the physicochemical properties are very similar. Both of these characteristics which cause separation difficulties to find saponins, and also the difficulty of determining its structure Furthermore, saponin triterpenoids also have a lot of diversity of and the molecular structure is more complex than steroid saponin. Saponin triterpenoid skeleton structure of the most dominant in nature are oleananes ( $\beta$ -Amyrin), ursine's ( $\alpha$ -Amyrin), and lupanes (*lupeol*). There is also a minor in nature are sapogenin taraxerol, taraxsterol, friedelin, glutinone, hopanes. Other triterpene saponin core framework is dammarane while commonly found in marine organisms is lanostanes and halostanes. Opportunities diversity of triterpenes saponins together with steroid saponin are huge the physicochemical properties are very similar to one and the other compounds.

In triterpene saponin with olean aglycone-12-en instance there are monodesmosidic sugar substituent or bidesmosidic or tridesmosidic or OH or COOH substituent or other substituents at position S or R or  $\alpha$  or  $\beta$  that can be bound to atom number C-1; C-2; C-3; C-16; C-22; C-23; C-19; C-21; C-24; C-29; C-6; C-23; C-28; C-30 (Hostettmann dan Marston, 1955). This situation illustrates the diversity of saponin olean-12-en very much with physicochemical properties are very similar to one and the other compounds. Opportunities diversity triterpene saponins also happen to be the core framework triterpene saponins 10 others with the same or different substituents both types of substituents and location of substituents.

Based on this phenomenon, saponins are very abundant in nature, but is hard to find in the form of pure compounds for chemical separation requires high-tech instruments. Rijai et al (2016) found a compound triterpene saponins from the seeds Kolowe (*Chydenanthus excelsus*) identified as a pure compound with chromatograms single peak by analytical HPLC, but the results of structure elucidation by NMR-2D frequency of 400 MHz on a variety of techniques, it turns out compounds that are considered pure are still the two compounds saponin which is a mixture of isomers. If the determination of the structure of saponins does with technical analysis without having to physically obtain compounds through the isolation of compounds by using LC-NMR, the compounds can not be tested in laboratory potential biological activity and difficult to modify the structure for potential development. Thus still very much needed isolation techniques saponin compounds to find compounds physically.

Based on the description, the saponins research issues of natural resources is the difficulty of separation to find saponin compound, when using the method or separation techniques such conventional column chromatography Gravity (normal phase, known as a method that is easy and inexpensive. Saponin compounds the difficulties of separation, there are many researchers, especially students do not understand it properly so that the saponin research is still being done by conventional methods and ultimately a failure after a long period. Potential pharmaceutical saponin in the pharmaceutical field as well as on the availability of natural resources that many, has become an attraction to the researchers, although the results failed because it has no chemical instruments for effective separation and chemical instruments for the determination of compounds through the determination of structures of saponin. Analytical and preparative HPLC instrument / semipreparative are a quick technique to obtain the compound saponin, though generally not all compounds identified by analytical HPLC can be separated by preparative HPLC or semipreparative. A compound saponin can be shown as an amorphous solid that resembles a pure compound with intervals of melting point 1-2 ° C, but with the use of NMR-2D frequency  $\geq 400$  MHz with a variety of techniques that compound was a mixture of isomeric compounds (Rijai et al., 2016).

#### METHOD

This scientific article is a review of various studies and books with a focus saponin The reference about the study on the technique of quickly isolation and structure elucidation of saponins compounds used by researchers. The results of the research which are the object of analysis is a range of scientific articles have been published, the results of research for the dissertation and thesis students, as well as The reference books separation and structure elucidation of saponins. The results of a major study on saponin which is the object of analysis is to Laode Rijai doctoral thesis (2004), Rumampuk doctoral thesis (2001), The reference book Saponin by Hostettmann and Marston (1995), Akihito et al., (2000); Gaidi et al. (2000a and 2000b), Herlt et al. (2002), Laode Rijai et al. (2016), Laode

Rijai (2016), Kazuo et al. (1999), Kinoshita et al. (2000), Maged et al. (2000), Rachman et al. (2000), Sanchez et al. (2000), Sadiqa et al. (1999); and Qiu et al. (2000). In addition to these sources also refer to the book The reference Saponins by Hostettmann and Marston, 1995).

### RESULT AND DISCUSSION

#### 1. Fast Extraction Technique for saponin

Extraction technique to obtain a saponin extract with no other metabolites have to do. This technique is very important to take advantage of saponin extract based on the potential, especially in the field of pharmacy. Fast extraction of effective saponin extract the most widely used solvent 96% distilled water-methanol ratio of 3: 7 or 1: 1 (Rijai, 2016). Extraction technique of saponins to obtain a saponin extract with no other metabolites (Rumampuk, 2001 and Rijai, 2004), which is a dry powder or fresh ingredients biological plant macerated with methanol-water (7: 3) or (1: 1) at room temperature is obtained a crude extract of plant samples. Frkasinasi can be done by using a liquid-liquid gradient from n-hexane-water; ethylacetate-water; n-butanol-water; or use a solid-liquid technique is silica gel with organic solvent gradient also is n-hexane, ethyl acetate, n-butanol, ethanol, methanol. The whole extract obtained, starting saponin extract ethyl acetate test using distilled water as the solvent with a foaming indicator that many until it reach a height of 30 cm. Extracts were shown to contain saponin based tests, followed by the extraction of saponins from the extract. Biological extracts taken sufficiently (10-30 g) and then dissolved in 96% methanol or methanol pro analysis (better) taste and shaken. The solution is added ether solvent to form a precipitate which is a saponin extract. The saponin extract can be obtained by means of filtration using filter paper or decantation. The extract obtained is a saponin extract, and can be tested again with distilled water or a solvent with an analytical HPLC analysis for comparison with HPLC chromatogram profile saponins have been there.

#### 2. Fast technique for Isolation Compounds of Saponins

Saponins are a class of natural compounds of biological highly polar stationary phase chromatography necessitating the non-polar or low-polarity for the separation, which is known as a reverse phase. Furthermore, the diversity of the many kinds of saponins with physicochemical properties of the compound and the other one very much like the main cause of the difficulty of separation of saponins with conventional separation techniques. the technique of isolation of saponins which are currently considered the most effective and rapid use of the stationary phase HPLC with a preparative column or semipreparative. Implementation of insulation saponins (Rijai 2004 and Rumampuk, 2001), are:

- a. Determination of solvent eluent for separation using analytical HPLC. A commonly used solvent for the separation of saponins is methanol-water or methanol-water-acetic acid specific to very polar saponins triterpene saponins substituted COOH. Comparison of analytical HPLC eluent was found with an eluent for separation with preparative HPLC or semipreparative.

The results of analytical HPLC separation with most good if at least use the detector Waters R410 RI compared with other like Waters 490E UV (Rijai 2004 and Rijai et al., 2016)

- Furthermore, a number of saponin extract is then diluted with eluent is separated by preparative HPLC / semipreparative column C-18 or C-8 reversed phase. The entire spectrum of analytical HPLC peak in the attention that is housed separately between the peak and the other one from the elution by preparative HPLC / semipreparative. The dominant peak is higher than the other peak in the spectrum of the compound quantitatively describe more than others. However cultivated all analytical HPLC peak in the spectrum is collected separately between the peak and the other one although not dominant.
- If the peak result of separation by preparative HPLC / semipreparative does not provide peak sharp single analytical HPLC and preparative HPLC, the fraction is still a mixture of compounds and should be a separation of the manner and the eluent same is by preparative HPLC or semipreparative to find an isolate pure. Examination of saponins purity using analytical HPLC.
- Saponins found the procedure a, b, and c have determined the structure by spectroscopic techniques FTIR, NMR, and MS or NMR and MS.

### 3. Structure Elucidation of Saponin

Characteristics of structure elucidation is a complex saponin, glycoside and form a structure conformation so it is possible many chemical signals are overlapping. Conventionally saponin structure determination by derivatization techniques using an enzyme or acid as hydrolyze to disconnect glycoside bond. This technique requires a considerable amount of saponin, whereas in general the amount of saponin obtained every saponin is very little insulation. Because of the structure elucidation of the most effective saponin compound, either steroid or triterpene saponins using  $^1\text{H}$  and  $^{13}\text{C}$  NMR instrument-2 D with a frequency  $\geq 400$  MHz and FAB-MS or ESI-MS (MS software). Characteristics of structure determination saponin aglycone consist of determining the type, number and type of sugar, the location of the glycoside bond, the bond between monosaccharides, and other substituents such as hydroxy, acyl, along with its location in the aglycone, and stereo or any position substituents. Saponin structure determination strategy, are:

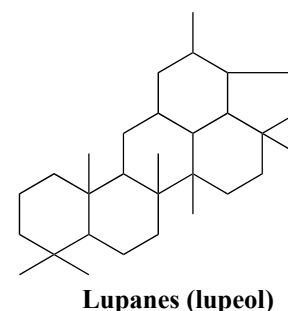
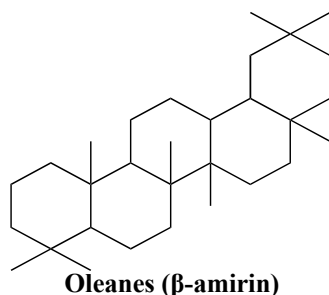
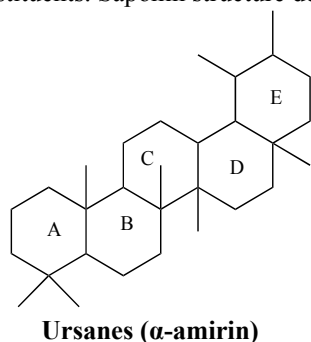


Figure 1. Aglycone triterpene major of main frame

#### a. Determination Compounds As saponin

Ascertainment of an isolate a compound saponin can be done by direct observation of physical properties of saponins as foam formation a lot in the water, the powder form is generally in the form of an amorphous solid, the style spectrum basis of  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR, style FTIR spectrum with absorption cyclic and sugar, as well as the masses MS results molecules that range  $\geq 546$  for steroid saponin with one part sugar molecule without other substituents and  $\geq 590$  for triterpene saponins lupana with one part sugar and also without other substituents (Rijai, 2004; Rumampuk, 2001; Rijai, 2016). Determination of saponin by chemical methods are dissolving the pure compound in water should be avoided because of the quantity or amount of saponins found in general very little, so the potential loss of the pure compound saponin.

Basic C NMR spectrum has been able to determine the number of atoms C a compound where there is no signal of the C atoms are overlapping or similar. Aglycone triterpene saponins have 30 C atoms and steroids 27 C atoms, so if spectrum C NMR of a compound showed signals carbon  $> 27$  for steroid saponins and  $> 30$  atom C for saponin triterpene indicate that the C atom other is a substituent such as sugar, hydroxyl, acyl, and another alkyl.

#### b. Determination Saponin types or Aglycones

Rapid determination of aglycon can use 1D NMR spectra. With steroid saponin aglycon will generate two signals proton chemical shifts at  $\delta$  0.2 to 2.0 ppm; whereas the triterpene aglycon produces 6-7 signals with the chemical shifts. Prediction is assisted by  $^{13}\text{C}$  NMR signals to determine steroid or triterpene aglycone are there is two signal to the aglycone methyl steroids and 5-7 to the aglycone triterpene methyl signal with chemical shift ranges from 10-35 ppm.  $^{13}\text{C}$  NMR signal in support of their second metin signal for steroid aglycone and 5-6 signal to the aglycone triterpene the chemical shift 30-45 ppm. Thus the steroid or triterpene aglycone has been determined by  $^1\text{H}$  and  $^{13}\text{C}$  NMR-1D.

#### c. Penentuan Kerangka Inti Saponin Triterpene

Triterpene aglycone main frame there are three that are found (major) that oleanes, ursanes, and lupanes; whereas the triterpene aglycone minor or rare is taraxerol, taraxsterol, friedelin, glutinone, hopanes and also dammarane. Additionally, commonly found in marine organisms or marine organisms is saponin lanostanes and halostanes. In these discussions, aimed at the general saponin found are ursana, Oleana, and lupana (Figure 1).

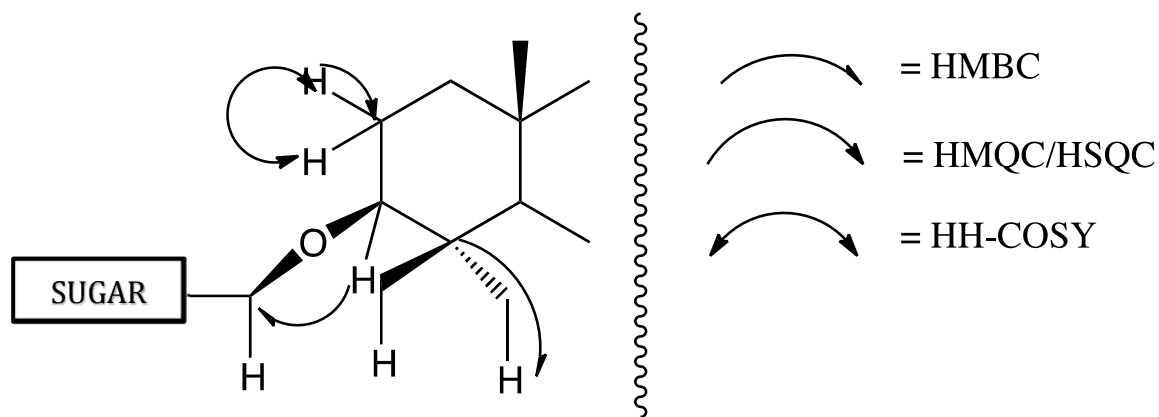


Figure 2. Structure determination for aglycone / saponin through glycosidic bond position

Core structure Oleana, ursana and lupana can be distinguished by signals proton NMR at atom C-29 and C-30, are on ursana will appear signal proton doublet-doublet with a constant coupling different, lupana form of double-doublet with constant coupling the same; while Oleana methyl proton signal at C-29 and C-30 in the form of singlet-singlet with a shift in chemistry from 0.5 to 1.5 ppm. Based on these data the core framework triterpene aglycon can be determined quickly using NMR techniques based on signal-1D proton and carbon. For a complete structure determination using NMR techniques should saponin HMBC, HMQC / HSQC, HH-COSY, ROE, ROESY and TOCSY. Positioning the C and H atoms aglycone begins with the observation of a dependent signal C atom that is part sugar experienced a chemical shift in the range 75-100 ppm and the signal must be confirmed by HMBC technique of observing the correlation signal C atom by atom H aglycone part sugar; and conversely signal atom C part sugar (usually 80-104 ppm) at H atom attached to the C atom aglycone. Based on these observations will be revealed C atoms aglycone signal a signal dependent part sugar and part of the C atoms of sugar bound to aglycone. The position of the sugar part of the aglycone was observed by constant coupling (coupling) anomers proton bonded to signal C aglycone, with range  $J = 6.5$  to 8 Hz showed a  $\beta$ -glycosidic position. The position of the glycosidic bond must be confirmed by the ROE and ROESY techniques. Furthermore, the position of C and H atoms in the structure of the aglycone is determined by using HMQC / HSQC to observe the correlation of the bonding between atoms C and H; HH-COSY technique for observing the correlation between H atoms bonded to one atom C. Thus shall aglycone structure determination using NMR spectrum of H and C NMR-1D; H and C NMR spectrum of 2-D techniques HMBC, HSQC / HMQC, HH-COSY, ROE, ROESY, and TOCSY.

Signal carbon prevalent at triterpene saponin range in chemical shift of 10 ppm to 200 ppm, especially if there are substituents carboxylic acid (COOH), containing monosaccharides glucuronic acid, and acyl substituents result in carboxylic acid esterification. Each atom C aglycone triterpene has already been recognized that if a paramagnetic chemical shift in the atom C was allegedly tied to more electronegative substituents. Therefore the whole atom C experiencing the paramagnetic chemical

shift of major concern in the determination of the structure that is determining the type of substituents including parts sugar. In addition, saponin consisting of ring A, B, C, D and E so that it will form four geometry ie A / B; B / C, C / D and D / E. The geometry of the ring may be in the form of trans and cis. Special saponin Oleana have trans geometry to A / B, B / C, C / D, while D / E can be trans or cis. Because it takes a certain technique to determine the geometry. The best technique to define the geometry of the aglycone ring is NMR techniques and ROESY ROE. ROE technique serves to determine the position of certain H on the ring through H correlation another ring with the position of a piece, while ROESY determines the position of the ring H and the other one with non-level positions. If the results of the analysis with these techniques, it can be determined that a ring has a trans geometry if not a plot, and cis if the plot. The determination of the geometry must be preceded by a hypothetical aglycone structure, as in figure 3.

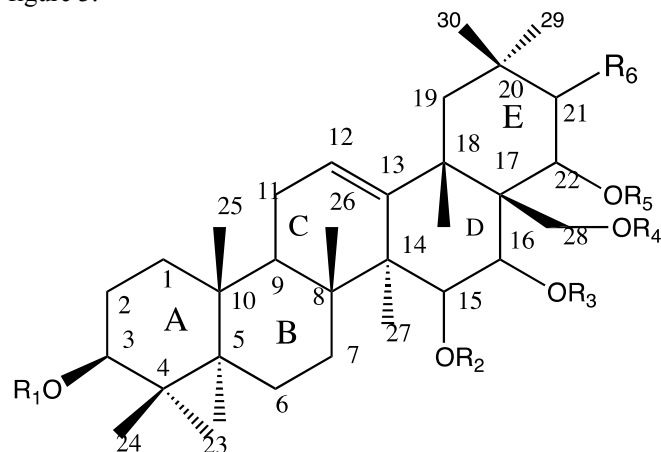


Figure 3. The structure of the hypothetical a saponin aglycone / saponin triterpene

Based on the hypothetical structure, the major concern is the structure R1 - R5 for signals in these positions must undergo a shift paramagnetic showing more electronegative substituents than others including R6 C atoms. If the C atom electronegative substituents aglycone without the chemical signal that indicated range at 23-30 ppm (Jiang et al., 1999). Determination of ring configuration and substituents did by using ROE and

ROESY. The determination of the geometry of A / B and ROE and ROESY experiment on proton H-25 and H-5; geometry B / C trial ROE and ROESY on H-26; geometry C / D with irradiated H-27, whereas the D / E with experimental H-28 and H-18. The technique is based on the geometry of A / B; B / C; C / D and D / E can be determined. For example geometry D / E to triterpene Oleana, atom C-28 is above the ring plane ( $\beta$ ) so that the C-28 to comparators in the trial ROE and ROESY. If the irradiation is given to H-18 and the result is an effect or no effect on the H-28. If the irradiation of H-18 shows the influence on the H-28, it is a field with HAB-28, which means the geometry of D / E is cis, and if it does not give effect to the opposite situation, namely D / E is trans. Cis position (Figure 4).

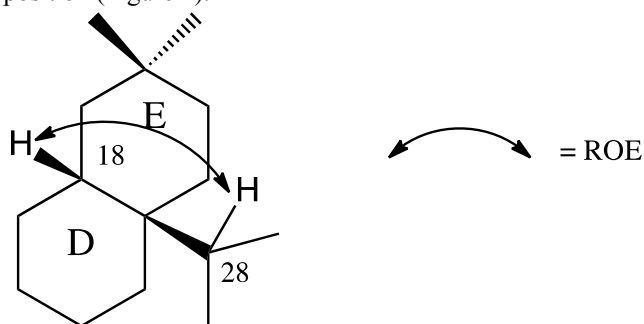


Figure 4. Determination of cis ring geometry D / E saponin triterpene

ROE experiments were also conducted on the configuration of substituents that can be determined appropriately. Conclusion aglycone of the hypothetical can be the figure 5.

#### d. Determination of acyl substituent

The acyl group is the result of the reaction of the hydroxyl substituent aglycon with carboxylate form acyl as an ester. Their esters have been indicated in the FTIR spectrum that is the uptake  $1306 - 1155 \text{ cm}^{-1}$  with weak intensity and forms a wide band, but the uptake may also indicate the presence of sugar, and piran ether so that more

information is needed. Ascertainment of their substituents acyl saponin is very important because it allows the determination of the amount of monosaccharides a saponin, which is based on the number of C atoms that have been identified through  $^{13}\text{C}$  NMR spectra 1D including DEPT techniques. Total atom C common steroid aglycone is 27 to aglycon colestan, furostan, and spirostan (saponins alkaloids), 26 for spirostan and solanidine (saponins alkaloids). Furthermore, the number of C atoms for common triterpene saponins is 30. If the number of C atoms by  $^{13}\text{C}$  NMR spectra 1D and DEPT > 30, then the excess is a substituent. If a triterpene saponins have 52 carbon atoms based on the analysis of  $^{13}\text{C}$  NMR spectrum of which there are atom C with a chemical shift of about 35 ppm indicate the C atoms that there are no substituents (Gaidi et al., 2000; Sanchez et al., 2000; Rahman et al., 2000), if there is a shift in the atom C with 60-65 ppm indicate the C atoms are hydroxyl substituent (Cadre et al., 2000). If a C aglycone substituted sugar or acid will have a greater shift paramagnetic because more electronegative than hydroxyl mainly sugar up to 185 ppm (Jiang et al., 1999), and, if substituted acids at the C substituents hydroxyl there will be a shift in the chemical atom C of the > 65 ppm and < 100 ppm (Qiu et al., 2000). The existence of an acyl substituent at C atom aglycone followed by HMBC techniques that correlate the distance between the aglycone signal H atom by atom C also acyl or C atom by atom H aglycone acyl substituent. If an acyl group as a substituent on a chemical shift, in general, have higher because more electronegative or a C carbonyl which is about 170-180 ppm (Qiu et al., 2000). Signal carbonyl C atom acyl proton signal correlated with a number of acyls ranged from 1.00 to 1.5 ppm (Qiu et al., 2000). Thus the structure of the acyl groups substituted on the C atom saponin aglycone can be determined either with 1D NMR spectra; HMBC NMR techniques; TOCSY, HH-COSY, and HMQC / HSQC.

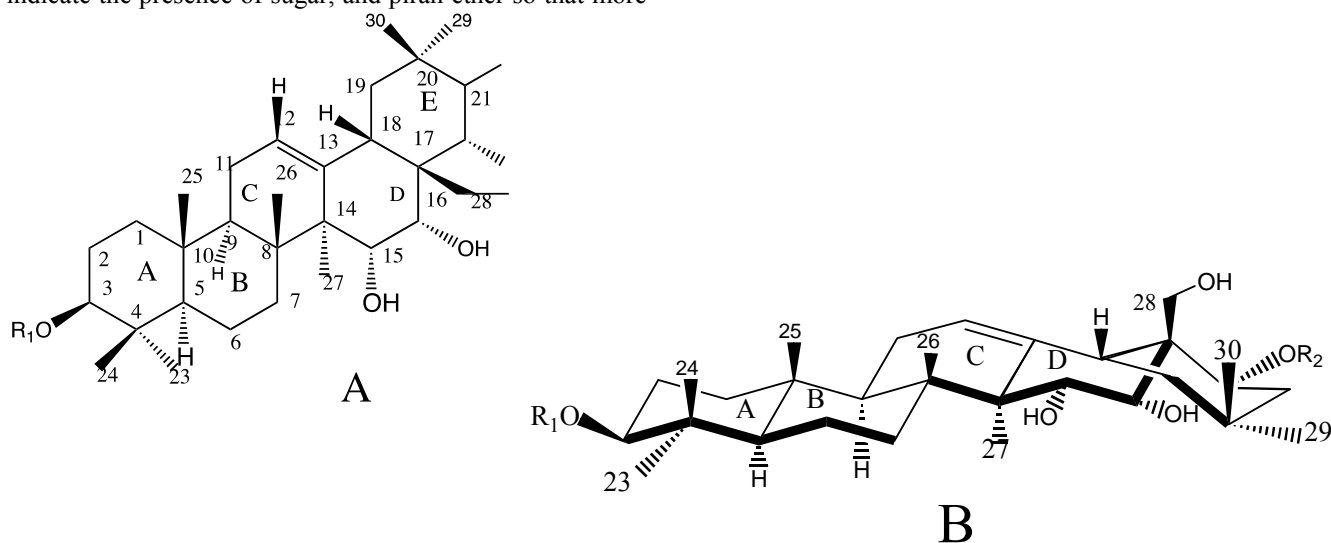


Figure 5. Determination technique of the geometry and conformation ring as well as the configuration of substituents on triterpene saponin Oleana

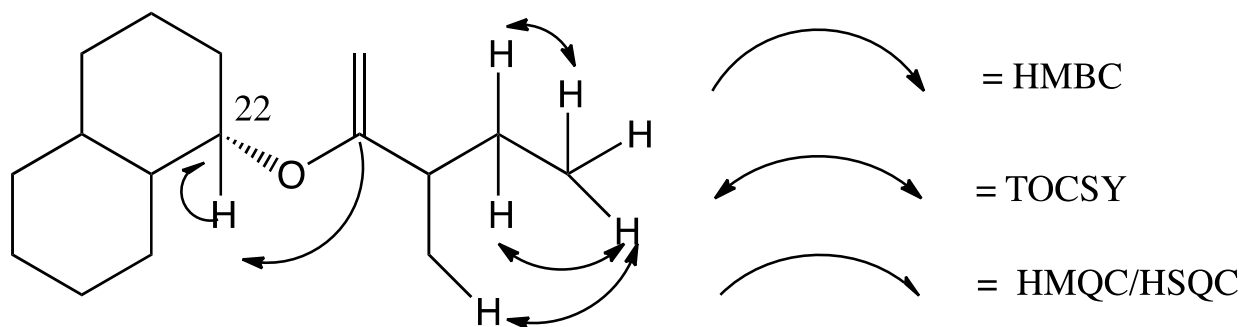


Figure 6. Acyl substituent structure determination technique of in saponins

#### e. Determination of Monosaccharides

Monosaccharides commonly found as part of saponin sugar is D-glucose, D-galactose, D-glucuronic acid, D-ribose, D-xylose, L-arabinose, L-fucose, and L-rhamnose. The parts of the sugar atom substituted at C-3 or more C atoms with  $\alpha$  or  $\beta$ -position anomeric. Parts sugar or monosaccharide that can be bound to different C atoms in one type of saponin. If the sugar part is only attached to the C atom is called monodesmosidic, on two different C atoms called the bidesmosidic, and three different C atoms called the tridesmosidic. Until now saponins found still limited to tridesmosidic and undiscovered tetradesmosidic.

Type of saponin monosaccharides are hexoses with 6 C atoms except D-glucuronic acid 7 atom C. In theory, if a triterpene saponin is bound to one molecule monosaccharide will find 36-37 signal atom C; if two sugar molecules are 42-43 signal atom C; and if three molecules will be found 48-49 atom signal C. That situation may occur if there is no signal of the C atoms are overlapping, so that the number of atoms C signal is not always used as a primary consideration. Determining the type starts with the determination of monosaccharides saponin aglycone a C atom bound monosaccharide and the next monosaccharide determination of C atoms in the atom C monosaccharide directly attached to the aglycone, and so on. Thus the determination of the monosaccharide and aglycone C atom bound monosaccharide place gradually. Monosaccharide bound to the C atom aglycone symbolize with M-1; monosaccharide bound to the M-1 symbolize with M-2 and so on. If saponin is bidesmosidic or tridesmosidic it can symbolize with M-2 'and M-3 "; whereas monosaccharide bound to the M-2 symbolize with M-2'-1; bound on M-2'-1 symbolizes with M-2'-1 'and so

on. The symbol can be made freely by the researchers in accordance with the level of convenience that were understood. Determination of atom C-section where the bound sugar next part sugar is a monosaccharide structure determination so well known as type and structure of the monosaccharide sugars as part of saponin.

#### 1) Determination of Monosaccharide M-1

Monosaccharides M-1 is the monosaccharide directly attached to the aglycone of saponins. Determination of the monosaccharide substituents using techniques HMBC by correlating signal aglycone a C atom bound with M-1 H atom monosaccharides, and the C atom at M-1 with the H atom attached to the C atom aglycone. The HMBC information has confirmed the existence of M-1 bound to the C atom aglycone. Configuration or position of the M-1 as a  $\beta$ -glycosidic or  $\alpha$ -glycosidic can be known through constant coupling proton atom C aglycone and the C-1 'in M-1, but must be confirmed by techniques ROE and ROESY for H atoms which are unidirectional or not unidirectional the sugar molecules, whereas TOCSY help correlate the influence of H atoms bonded to atom C aglycone with H atoms bonded to atom C sugar or between the H atoms bonded to atoms of C part sugar is by irradiation H atoms. Thus the NMR technique is important for determining the structure of monosaccharides M-1 is HMBC, HMQC / HSQC, HH-COSY, ROE and ROESY, TOCSY. Signal atom C sugar section generally ranges in chemical shift 65-110 ppm; while the proton signal ranges from 3.3 to 5.0 ppm (Hostettmann and Marston, 1995). If there is a signal > 170 ppm should be assumed as a carboxyl C atoms, such as glucuronic acid. The presence of carboxyl groups can also be identified on FTIR absorption as C = O and C-O-C and C-OH carboxyl.

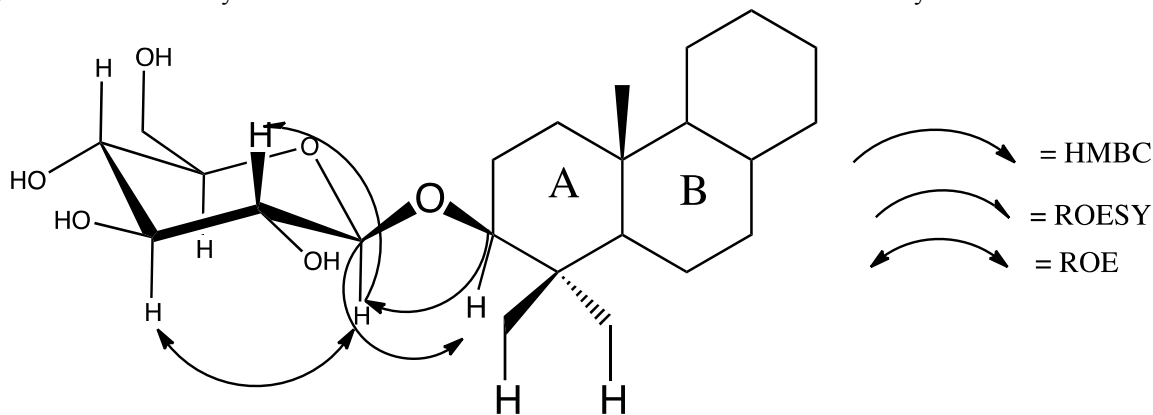


Figure 7. determination technique of the structure of monosaccharides M-1 saponin

## 2) Determination of Monosaccharide M-2

Monosaccharides M-2 is a monosaccharide molecule bound to the monosaccharide M-1. Possible ties between M-1 and M-2 can be either  $\beta$  or  $\alpha$  position to form  $1' \rightarrow 2''$  or  $1' \rightarrow 3''$ . NMR techniques of are mainly used to determine the structure and configuration of the M-2 is HMBC, ROE, ROESY, TOCSY enhanced with HH-COSY, HMQC / HSQC. Signal carbon commonly found also range in a chemical shift at 65-110 ppm; while the proton signal ranges from 3.3 to 5.0 ppm (Hostettmann and Marston, 1995). Determining the structure of M-2 is equal to monosaccharide M-3, and so if part sugar saponin consists of 3 or 4 molecules of monosaccharides.

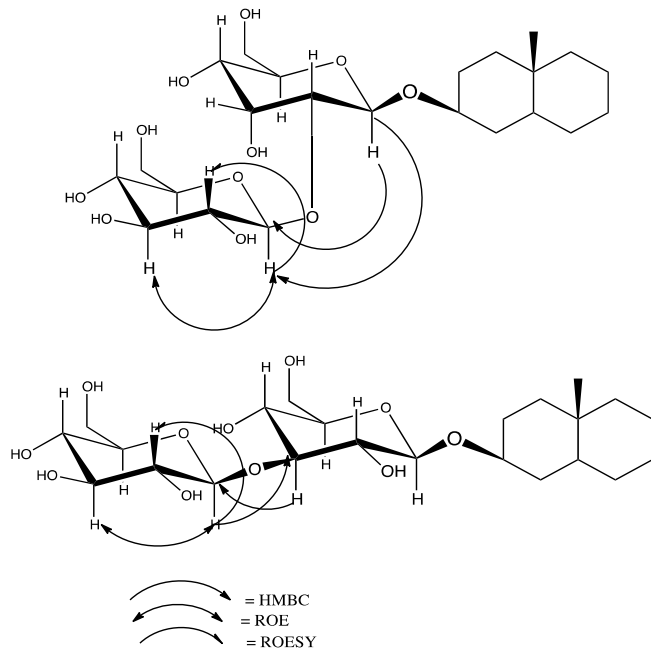


Figure 8. Determination technique for monosaccharides M-2

## CONCLUSION

1. Fast technique saponin extraction using methanol-water with a ratio of 7: 3 or 1: 1 and perform the purification of saponin mixture (without other metabolites) using ether were added in a solution containing saponin fractions.
2. Mechanical precise and rapid isolation of saponins are using preparative HPLC column or semi-preparative HPLC eluent with determining the analytical column
3. The appropriate eluent for the separation of saponins by reverse phase technique using preparative HPLC column or semipreparative are methanol-water or methanol-water-acetic acid to very polar saponins
4. Engineering quick determination of the structure of saponin in a way to structure elucidation stages, namely the determination of the aglycone, the determination of the sugar portion consisting of monosaccharides attached to the aglycone, monosaccharides bound monosaccharides attached to the aglycone and so on, as well as the determination of substituents commonly found in saponins such as methyl, metin, acyl, and hydroxyl

5. The main instrument for structure determination is a saponin-2D NMR frequency  $\geq 400$  MHz with techniques HMBC, HMQC / HSQC, TOCSY, ROE, ROESY, and software such as MS instruments FABMS (ESIMS).
6. NMR techniques in addition to those techniques are also considered very helpful is the DEPT spectrum and NMR-1D
7. FTIR instrument helped to accelerate the general determination that the identified compound is a steroidal glycoside or triterpene glycosides

## RECCOMENDATION

Isolation and structure determination of saponin from a natural material of biological directly using extraction techniques that, using preparative HPLC or semipreparative and analytical HPLC for the determination of eluent, while the determination of the structure directly using NMR-2D with a frequency  $\geq 400$  MHz with techniques HMBC, HMQC / HSQC, ROE, ROESY, TOCSY, and HH-COSY. Such techniques may be assisted by 1D NMR spectra and DEPT techniques, while another important instrument is the MS with FABMS (ESIMS) and FTIR.

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This article aims to present a technique of isolation and structure elucidation saponins precise, fast and effective, due to the isolation and structure elucidation of saponins known to be very difficult despite the use of separation equipment and high-tech instruments. Therefore I as a reviewer of this article would like to thank all the writers of books reference and author of the scientific article that became the source of this scientific article. The authors are those listed in the bibliography of scientific articles and are also written in the methods section of this article. Hopefully this article useful to researchers saponin especially the students and the services of the authors of articles and books as a source of this article.

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