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Literature Review: Analysis of Saponins in Several Plants by HPLC Method and Its Utilization in Pharmaceutical Products

Vony Intan Prasasty[1]

[1]*Universitas 17 Agustus 1945 Jakarta*

**Corresponding Author: Vony Intan Prasasty*
E-mail: vonyintan05@gmail.com

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Abstract

This research aims to determine the content of saponin compounds found in several types of plants and their benefits for health. This research uses computer equipment and the Google Scholar database, Research Gate, NJC journal, Science Direct. The type of research used is an article review using data sources directly collected by the researcher. The data search was carried out through a Google Scholar database, using the keywords "saponin levels and HPLC". The screening results that met the requirements were used as references for 25 articles. This research used plant samples using the dried leaves of the plant and then extracted using the ultrasonication method, then using centrifugation to obtain a clear solution. The saponin analysis of the samples was carried out. Using HPLC with various columns. From these results, the saponin compound was shown to appear at a retention time of 53.09 minutes with an avarage saponin amount of 1.05%. Pharmacological studies show that saponins have many important such as enhancing immunity, antitumor, anti-inflammatory, antifungal, and anti-viral actions, reducing blood glucose and lipids, antioxidants, improving cardiovascular function. From the results of research conducted by several researchers above, it can be concluded that saponin analysis in several plants can be carried out using HPLC with a wavelength of 205nm. Saponin compounds as secondary metabolites have many benefits in the pharmaceutical field, including anti-inflammatory, hepatoprotective activity, immunological improvement, anti-tumor, anti-aging, treatment of cerebral edema, lung injury, myocardial ischemia reperfusion injury and gastric ulcers.

Introduction

Saponins are one of the secondary metabolites produced by plants (Goral, 2020). A common feature of the structure of saponins is the presence of a triterpene or steroid aglikone part, usually characterized by one or two oligosugar groups, then linked to the aglikone through an ester bond (Goral, 2020). Saponins are included in the group of active compounds that are used as natural surfactants derived from plants. Saponacuous substances can be shaped like soap with water-soluble foam, so they can be referred to as hydrophobic and hydrophilic molecules (Nafiunisa et al., 2019).

Saponins are important products that are produced naturally and were first found in plants, followed by several marine organs such as sea cucumbers and coral flowers (Safarino, 2021). The results of a review of 40 journals obtained more than 1730 species of Asian plants, with 76% containing plant and vegetable saponins consumed for diet programs (Safarino, 2021). Research conducted by (Sharma et al., 2020) Saponins have biological activity as medicinal

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properties, antimicrobial activity, antiviral activity, and others. Saponins have natural potential value as an antiviral against Cov-19 (Sharma et al., 2020).

Quantitative analysis of saponin content in plants can be carried out by the HPLC method with ultraviolet light (UV) detectors, diode aray detectors (DAD), light evaporative detectors (ELSD), aerosol-charged detectors (CAD), and mass spectrometry (MS) (Vu-Huynh et al., 2020). HPLC is so far considered as a common separation and analysis emtode for the quantitative determination of saponin content in plant species, this is because HPLC has the advantage of convenience and has a fairly good level of accuracy (Nguyen et al., 2021). This study uses an article review method or literature study on the levels of saponin compounds in several plants. The reason for conducting this study is to see the difference in the levels of saponin compounds found in the leaves of each plant.

Methods

Tool

The tools used in this article review research are computer devices and Google scholar databases, research gates, NJC journals, Science direct.

Working Procedure

The type of research used is an article review using data sources that are directly collected by the researcher. The data search was carried out through a database using Google scholar and obtained 40 articles published from 2015-2023.using the keywords "saponin levels and HPLC". The results of the qualifying screening were used as a reference for 25 articles.

Result and Discussion

From the results of research conducted by several researchers, and presented in the form of journals, which are then selected to make literature review, the results are presented in the form of table 1 below.

Table 1. Levels of saponin compounds in the leaves of some plants with HPLC

No	Source	Plant name	Number of samples	Saponin as much as Hasil Analisis
1	Mikhail Avsef (2021)	Ginseng	2000ng/ml	Saponins detected
2	Ying Zhao (2020)	The	12.5ml	Saponins detected
3	(Król-Kogus, Barbara, Daniel Głód, 2020)	Biji fenugreek	5 g	Saponins detected
4	(Vu-Huynh et al., 2020)	Panax vietnamensis	100mg-	Saponins detected
5	(Nguyen et al., 2021)	Panax vietnamensis	100mg	Saponins detected
6	(Qian et al., 2022)	Camellia oleifera	20mL	Saponins detected
7	(Savarino, 2023)	Tribulus terrestris L. fruits	0.5 g	Saponins detected
8	Miao Yu (2015)	Hedera helix L.	0.2mm	Saponins detected
9	Katarzyna (2016)	Beta vulgaris L	100g	Saponins detected
10	(Onlam, Suran, 2017)	Asparagus racemosus	100g	Saponins detected
11	(Bajad et al., 2019)	Dodonaea viscosa	10g	Saponins detected
12	(Chen et al., 2021)	Sapindus	10g	Saponins detected
13	(Roonjho et al., 2022)	Porterandia anisophylla Antidesma cuspidatum	15µl	Saponins detected

		Clidemia hirta		
14	(Yasmeen et al., 2022)	Hedera Heli	500mg	Saponins detected
15	(Wang et al., 2021)	Ginseng liar Forest ginseng Cultivated ginseng	100mg	Saponins detected
16	(Kiuchi, 2022)	Aakar Accharanthes	Not mentioned	Saponins detected
17	(Amirah, 2024)	Nephelium lappaceum	100mg	Saponins detected
18	(Chen et al., 2022)	Pulsatilla chinensis	20mg	Saponins detected
19	Min Lia (2020)	Panacis Majoris	1kg	Saponins detected
20	(Roonjho et al., 2021)	Phaleria macrocarpa	100g	Saponins detected
21	(Sun et al., 2019)	Quinoa	50g	Saponins detected
22	(Sharma et al., 2020)	Chlorophytum Borivilianum	20mg	Saponins detected
23	(Vijendra, Poornima D., 2020)	Leucas aspera Spreng	500mg	Saponins detected
24	(Tlili et al., 2021)	Rhus tripartite	20g	Saponins detected
25	(Narvaez et al., 2023)	Unguiculata vineyard	10kg	Saponins detected

Mio yu (2015) in his study used a sample of the Hedera helix L. plant by using the leaves of the plant that had been dried and then extracted using the ultrasonication method using Bransonic 5510, then by using centrifugation to obtain a clear solution, then the analysis of saponins in the sample was carried out using HPLC with various columns. In this analysis, the motion phase used in the form of a mixture of water and acetonitrile combined with several mixtures of phosphoric acid, acetic acid, and boric acid was used to increase the resolution and remove the tailings peak from the target compound. From these results, saponin compounds were shown to appear at a retention time of 53.09 minutes with a number of saponins of 1.05%.

The analysis of saponins in the following plants was also carried out by Katarzyna (2016), using a sample of Beta vulgaris plants as much as 100 grams. The samples were extracted using the ultrasonic method for 7 hours, the results of the viscous extracts were fractionated and produced a clear residue. The residue that has been obtained is then analyzed using HPLC using the phase of motion of formic acid and acetonitrile, with a flow rate of 200 ÿL/min. The results obtained in this study included 26 peaks that showed the saponin group compounds in the plant (figure 1).

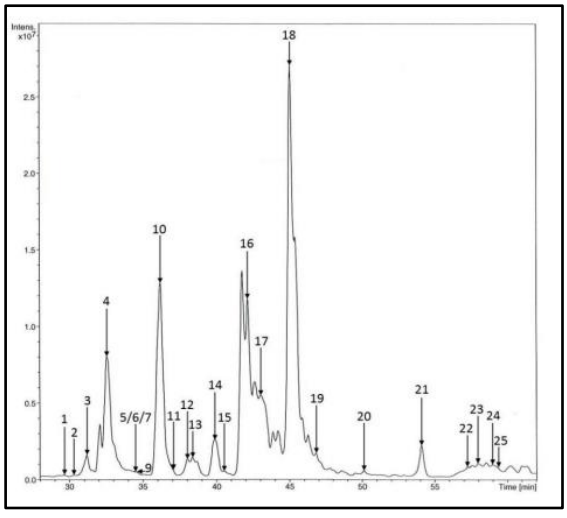

Figure 1. Results of saponin analysis using HPLC (Katarzyna, 2016)

According to (Onlam, Suran, 2017) In his research, he used samples *Asparagus racemosus* with a sample size of 100 grams. The samples were collected from several provinces of Bangkok, Phetchabun, Phitsanulok and Rayong in Thailand, then the samples were extracted using sonication in TranssonicTP690 (Elma, Germany) for 15 minutes with maximum power, filtered through a 0.45 µm syringe filter and injected (10 µL) then into the HPLC. Conditions in the study used SecurityGuardTM C8 protective column (100Å, 5µm, 4 x 3 mm, Phenomenex, USA) and the column temperature was maintained at 35 ºC. The motion phase consisted of 0.1% formic acid in water (eluene A) and 0.1% formic acid in acetonitrile (eluene B). The gradient program is: 50:50% Eluen A/B at the beginning, then 40:60% A:B for 5 minutes, then linearly lowered to 10:90% (A/B) for the next 4 minutes, and held for 3 minutes with a flow rate of 0.6 mL/min. The results of this study show that the standard curve of the five saponin glycosides has an acceptable linearity in the range of 2.50 - 30.00 µg/mL with a correlation coefficient exceeding 0.997. The minimum concentration levels at which analysts can be reliably detected (LODs) and measured (LOQs) are 5 to 10 ng and 25 to 50 ng, respectively. The % intra- and inter-day precision RSD is less than 4% and 6%. Each of the samels is considered acceptable according to Horwitz's equation [20] (for an analyst at a concentration of 6 µg/mL, the %RSD should be <11 and for an analyst of 25 µg/mL, the RSD should be <10). For recovery the overall accuracy ranges from 95% to 105%, with an approximate RSD between 0.7-4.5% being within acceptable values.

Bajad et al. (2019) also conducted research related to saponins using *Dodonaea viscosa* samples with a sample of 20 grams of fine powder used. Sample extraction uses ethanol, methanol and water solvents using the 24-hour soxhletation method. The analysis of saponins in this study using HPLC produced saponins showing a peak at 205 nm which was matched with a standard saponin confirming the presence of saponins in isolated saponin samples from Dodonaea viscosa. The number of saponins present in Dodonaea viscosa was calculated and the % of saponins found was 173.25µg/mg.

The next research related to saponins is a study conducted by Chena et al. (2021) using fruit peel samples *of Sapindus mukorossi Gaertn.* The sample used was 10 g of powder sample. The sample was then purified using rehydrated yeast in 50 ml of saponin medium for 20 minutes. Saponin analysis using HPLC with a wavelength of 205 nm using 24 columns Symmetry C18 (250×4.6 mm), Sunfire C18 (250×4.6 mm) and Atlantis T3 C18 (250×4.6 mm) were used. Elucidate the analyte gradient using methanol (A) and water (B) with a final ratio A–B (84:16, v/v). The temperature of column 116 is maintained at 35°C, and the flow rate is 0.5 ml/min for 20 minutes. The results showed that there was a peak with the same condition between the saponin standard and the saponin in the sample. The results of the study are presented in the form of a chromatogram in the figure (2).

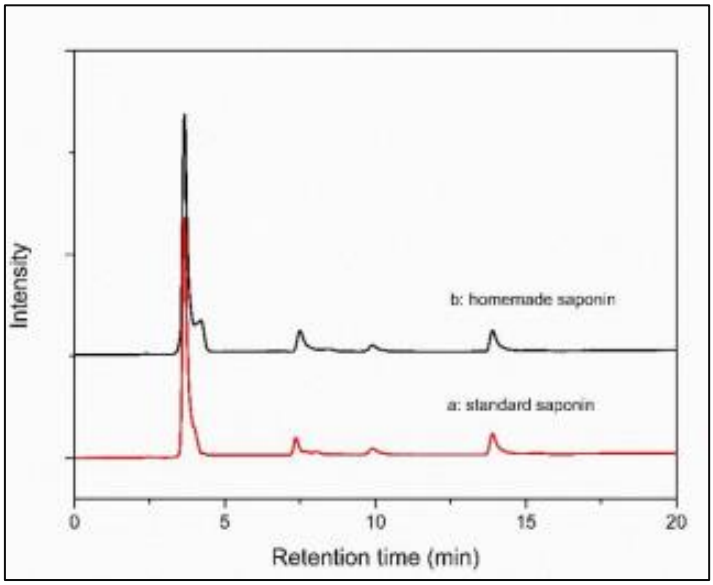

Figure 2. Results of saponin analysis using HPLC (Chena et al., 2021)

Saponins are secondary metabolites in plants with various pharmacological effects. Saponins with their pharmacological effects as described by (Binesh et al., 2018) in Chunlian Tiana (2020) said that saponins can be used as anti-inflammatory, have hepatoprotective activity, improve immunology, anti-tumor, anti-aging, treatment of celebral edema, lung injury, myocardial ischemia reperfusion injury and peptic ulcer. Jung et al. (2010) in Chunlian Tiana (2020), proved anti-inflammatory diosgenin by effectively inhibiting the secretion of IL-1, IL-6, NO and ROS in LPS and IFN-ÿ-induced mouse macrophages. Ginsenoside Rg1 can decrease the content of the cytokine TNF-ÿ and the NO inflammatory mediator, as well as increase the regulation of glucocorticoid receptor expression by dose-dependent.

Saponins in addition to having pharmacological effects also have benefits as pest repellents, Chaleb (2010) and Singh and Kaur (2018) in (Roonjho et al., 2022) revealed that saponins have the effects of inhibiting growth, preventing eating, cytotoxic and insecticide activity. Pharmacological studies show that saponins have many important biological functions of pharmacological activity and action (Jun, 2019), such as increased immunity, antitumor, anti-inflammatory, antifungal, and anti-viral action, reduction of blood glucose and lipids, antioxidants, improvement of cardiovascular function (Yingying et al., 2015) haemolysis (Savarino, 2023). In recent years, saponins have been widely used in medicines, health foods, animal feed, cosmetics, and other items. In addition, saponins are used as plant growth regulators and insect repellents in agriculture. Here are some plants with saponin content and their benefits in table 1 (Yi, 2022).

Table 2. Some plants with saponous content and their benefits

No	Plant Name	Plant parts	Contents	Benefit
1	*Panax ginseng C.*	Roots and rhizomes	*Triterpenoid saponins, ginseng polysaccharides, ginseng alkynols, amino acid proteins, sugars, vitamins, organic acids, trace elements, flavonoids and peptides*	Improve vitality, immunity, maintain lung and liver health.
2	*Panax pseudoginseng notoginseng(Burkill)*	Roots, leaves, flowers, rhizomes	*Dammar type tetracyclic Triterpenes*	Diffuse stasis to stop bleeding, reduce swelling and

			saponins, flavonoids, notoginsenosides (amino acids), proteins, volatile oils, acetylenes, alcohols, polysaccharides, polyols, polyacetylene alcohols, organic acids, trace elements, etc.	Pain relief
3	*Platycodon Grandifloras*	Want	*Oleanane-type pentacyclic triterpene saponins, flavonoids, phenols, sterols, polysaccharides, polyacetylenes, steroids, phenolic acids, polysaccharides, fatty acids, fatty acids and trace elements, inorganic elements, etc.*	Protects the lungs, relieves asthma, eliminate cold, beneficial for the pharynx, phlegm that comes out, removing pus, induce drugs Increase
4	*Astragali Radix*	Want	*Triterpene saponins, polysaccharides, flavonoids, amino acids*	antiperspirant, diuretic poisoning, releasing pus, collecting sore muscles, Benefits of water to reduce swelling
5	*Anemarrhena asphodeloides Bunge*	Rimpang	*Steroid saponins diphenpyrone, flavonoids, lignin, polysaccharides, alkaloids, amino acids, volatile oils, organic acids and trace elements, inorganic elements, etc.*	Heat cleaning and cleans the fire, nourishes Yin and moisturize Drought, cooling wear and eliminate distractions
6	*Bupleuri Radix*	Dry roots and grass	*I'm so grateful, flavonoids, volatile oils, polysaccharides, sterols, polyols, coumarins, lignans, fatty acids (oleic acid, linolenic acid, palmitic acid, stearic acid, etc.), tryptophan, wood sugar alcohol, uridine, adenosine and trace elements, etc.*	Antipyretic, anti-inflammatory, lowers blood cholesterol calms the heart and stagnant depression, Raising and lifting depression, protect liver and Cool, Relieve stasis hati, elimination, relief pain and reduced peradangan, anticancer, against the liver fibrosis, evacuation fever
7	*Polygala tenuifolia*	Want	*Triterpene saponins,*	Removes phlegm,

			ketones, oligosaccharides, alkaloids, phenylpropanoid flavonoids, lactones, coumarins, lignin, etc.	reduce swelling, calming the mind and Increases intelligence
8	*Glycyrrhiza ur alensis*	Roots and rhizomes	*Triterpenoid saponins Flavonoids and polysaccharides*	Repairs the spleen and dissipates heat and detoxify, expel phlegm and cough, relieves pain, reconcile all medications

Conclusion

From the results of research conducted by several researchers above, it can be concluded that saponin analysis in several plants can be done using HPLC with a wavelength of 205nm. Peaks with sufficient time retention are relatively varied according to the conditions of the motion phase.

Saponin compounds as secondary metabolites have many benefits in the pharmaceutical field, including, anti-inflammatory, hepatoprotective activity, improved immunology, anti-tumor, anti-aging, treatment of cerebral edema, lung injury, reperfusion injury of myocardial ischemia and peptic ulcer. In recent years, saponins have been widely used in medicines, health foods, animal feed, cosmetics, and other items. In addition, saponins are used as plant growth regulators and insect repellents in agriculture.

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