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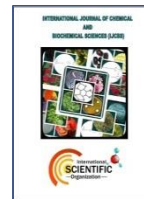


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Characteristics, analysis, and potential applications of saponin as a biological surfactant from *Nephelium lappaceum* (Rambutan) leaves: A mini review

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Abstract

Saponins are naturally occurring glucoside compounds found in various plant species, including the leaves of the rambutan tree. With their amphipathic structure, saponins possess a hydrophilic head and hydrophobic tails, making them capable of lowering surface tension and exhibiting solubilizing and emulsifying properties. As the demand for synthetic surfactants in diverse applications such as detergents, cosmetics, and industrial processes continues to rise, there is some interest in exploring alternative sources of surfactants. This mini review article aims to provide a comprehensive understanding of the saponins, including their characteristics, qualitative and quantitative determination, and chromatography analysis methods. Furthermore, exploring the potential applications of saponins as a biological surfactant derived from rambutan leaves, highlighting their unique properties and benefits over synthetic alternatives.

Keywords: Saponin, Rambutan leaves, surfactant, biosurfactants.

Mini-review article *Corresponding Author, e-mail: maiza@ump.edu.my

1. Introduction

Surface-active agents referred to as surfactants are amphiphilic or amphipathic molecules made up of a polar or ionic component that is hydrophilic and a non-polar hydrophobic section, typically a straight or branching hydrocarbon or fluorocarbon chain with 8–18 carbon atoms. The term "amphiphilic" origin from the Greek word "amphi," denoting "both," and it refers to the fact that all surfactant molecules have at least two portions, one of which is soluble in a particular fluid, such as water, and the other of which is not. The hydrophilic component might be both ionic and nonionic or nonionic, ionic, or zwitterionic¹. In an aquatic environment, the hydrocarbon chain interacts with water molecules rather weakly, as opposed to polar or ionic interactions. Surfactant, also stated as a surface-active agent, a material such as a detergent that decreases its surface tension when applied to a liquid, thus enhancing its dissemination and wetting properties [1-2]. In the general sense, surfactants may be considered to be any substance affecting interfacial surface tension, but in the practical sense, Surfactants have multiple uses such as dispersants, foaming agents, emulsifiers, and wetting agents. The surface molecule needs to be partially soluble in lipids, or oils, and partially hydrophilic (water-soluble) and partially lipophilic (water-

soluble). It concentrates at the interfaces where drops or masses of water meet lipids or oil as an emulsifying or foaming agent. Additional surfactants that are less hydrophilic and more lipophilic can be employed as demulsifiers or defoaming agents. Germicides, fungicides, and insecticides are certain surfactants. In corrosion inhibition, surfactants are used to facilitate the flow of oil into porous rocks and to create aerosols. Surfactants can be assigned into two, which are synthetic surfactants and natural or biosurfactants. Synthetic surfactant is commonly used in soap or detergent because of the better result. But the toxicity of synthetic surfactants is very high [3-6]. In this review, we provide a summary of the structural and empirical characteristics of saponins, which are naturally occurring surfactants derived from plants. We also highlight the qualities of these surfactants, which are comparable to those of conventional surfactants, as well as some possible uses for them [7-12]. Here, we also go over the latest developments, potential, and difficulties related to the creation of manufactured development based on saponins. Consequently, additional research is required to create industrial formulations based on saponins that can take the place of synthetic alternatives, utilising organic resources

more effectively and helping to create a more environmentally friendly world [13-16].

1.1. Comparative Analysis of Surfactants

1.1.1 Chemical Characteristics

In general, saponins are characterized as unstable, surface-active substances that are found widely in nature, mostly in the kingdom of plants [17]. Because saponin molecules produce foams that resemble soap when shaken with water, the term "saponin" comes from the Latin word *sapo*, which refers to "soap." Saponin are glycosylated substances made up of two primary components: a structure that is liposoluble and a water-soluble glucosidic chain. Fig.1 depicts the saponin's structural composition. Aglycone and glycone are the names of the sugar and non-sugar components, respectively. A triterpenoid or steroid backbone constitutes the aglycone part. Among the sugars are L-arabinose, D-xylose, D-glucose, D-glucuronic acid, D-galactose, L-rhamnose, and D-fructose are the components in saponin. From an ester or ether glycosidic bond at one or two glycosylation sites, the sugar moiety is connected to the aglycone. One or more unsaturated C-C bonds may be present in the aglycone. Monodesmosidic saponin have an additional sugar moiety at the C3 position of the oligosaccharide chain, whereas bidesmosidic saponin have an additional sugar moiety at the C26 or C28 position. The kinds and amounts of sugars, in addition to the steroid ring's structure, affect the structure of saponin from various plants. Young plants have been found to contain more saponin than mature or old plants, even though several factors, including physiological status and environmental factors, influence the saponin levels. According to the type of their aglycone, saponin are divided into two main groups: saponifies with steroidal aglycone and aglycones made of triterpenic saponifies [18]. The triterpenoid saponin retains all 30 C-atoms, but the steroid saponin has three methyl groups removed, making these molecules with 27 C-atoms. This is the difference between the two classes. They are made up of one or more monosaccharide moieties joined to non-polar aglycones. Their behaviors in aqueous solutions are similar to soap because of the constituent of polar and non-polar structural components in their molecules. Triterpenoid and steroid glycosides, or triterpenoid, spirostanol, and furostanol saponin, are composed of glycosyl residues linked to a skeleton formed from the 30-carbon precursor oxidosqualene [6].

1.1.2. Chemical surfactant

Surfactants can be biologically derived or manufactured. Synthetic or chemical surfactants are defined as those originating from synthetic materials, such as agricultural waste; biosurfactants, on the other hand, are defined as those originating from biological materials, such as living cells like bacteria, yeast, and fungi [10]. Numerous studies indicate that because synthetic surfactants are resistive and persistent, they may exacerbate toxicological and environmental issues. Synthetic surfactants are thought to be a powerful organic contaminant in soil, according to [6]. The texture, colour, and growth of plants are impacted by the accumulation of synthetic surfactants, which are currently utilised in pesticide formulation. These surfactants include anionic, cationic, amphoteric, and non-ionic. Since pesticide residues are known to linger in soil for years, move via the air

and water, and even remain on the surface of fruits and vegetables, these dangerous pesticides also leak into groundwater [20]. Because the majority of surfactants used daily such as detergent are chemically manufactured, they are hazardous to freshwater life. Since synthetic surfactants are mostly derived from petroleum, they are typically not biodegradable and hence continue to be hazardous to the environment. They could build up, and the environment might be harmed by their production methods and byproducts. According to a different study, triton X-100, a synthetic surfactant, is non-biodegradable in anaerobic environments and somewhat biodegradable in aerobic ones. In contrast, rhamnolipid biosurfactants are biodegradable in both scenarios [9]. In contrast to synthetic surfactants, biosurfactants sourced from renewable resources have superior surface activity, are non-toxic, biodegradable, have high specificity, display efficacy in unpleasant environments, and may be used multiple times during their creation. They are thought to be less hazardous, environmentally benign, and suitable for manufacturing uses in the food, cosmetics, pharmaceutical, and petroleum domestic [10].

1.1.3. Biosurfactant

Additionally, environmental isolates' biosurfactants show promise for use in the agriculture sector. Strong lipopeptide biosurfactant "surfactin" is discussed as a multifunctional bioactive agent that can prevent the formation of fibrin clots and be used for improved oil recovery. Its antifungal, antiviral, anticancer, insecticidal, and anti-mycoplasma properties have also been established. Additionally, surfactin can be applied to soil and water as a bioremediation agent [20]. Research has demonstrated that biosurfactants contribute to the decrease in greenhouse gas emissions. Oil chemical surfactant manufacturing prevented over 1.5 million tonnes of CO₂ emissions in 1998. A sustainable environment and agriculture will help to lessen the negative effects of synthetic surfactants, which are widely used in many commercial sectors. Given the varied applications of surfactants in the pesticide and agrochemical industries, food industries, pharmaceutical, and other sectors, it is necessary to use the more environmentally safe biosurfactants in these industries.

1.1.4. Saponin

Natural sources such as plants, bacteria, or fungi are the direct source of natural surfactants. The most well-known plant-based surfactant is saponin, which is a glycosidic molecule with a hydrophobic nucleus that is either an alkaloid steroid or a triterpenoid [14]. It has been discovered that the distribution of saponin varies throughout plant sections [19]. Nonetheless, it has been noted that leaves have the highest content of total saponin. Because they have a water-soluble sugar chain, a lipid-soluble aglycone, and an amphiphilic character, saponins are surface-active chemicals with detergent, wetting, emulsifying, and foaming qualities. Saponin has been commonly used for centuries as a cleansing agent. In stable, soap-like foams, their name is derived from their ability to form aqueous solutions. Structurally, saponin are amphiphilic compounds made up of one or more components of hydrophilic sugar and a part of a lipophilic steroid or triterpene (sapogenin). Other substances that are structurally closely related to saponin are often referred to as saponin, such as cardiotonic heterosides or glycoalkaloids.

These substances are treated similarly since their structures and effects on membranes are identical. Saponins are divided depends on the number of sugar chains that have one, two, or more, respectively, into monodesmosidic, bidesmosidic, and polydesmosidic saponins [18]. Due to the existence of various sugars, sugar branches, and sapogenins, a wide structural variety of saponins can be found in nature. Saponin can be found in many plants such as bunga cina leaves, ginseng, soap bark tree and others. [13] found eight new triterpenoid saponin with antioxidant activity from the roots of Glycyrrhiza while another study discovered that ginseng saponin was highly effective at reducing the interfacial tension at the soybean oil–water interfaces, and was capable of producing nano-scaled droplets Saponin also can be found in rambutan seed or leaves [9]. The seeds of rambutan are bitter and stated to have narcotic effects since the seed contains traces of alkaloids and the test contains saponin and tannin. A high intake of saponin and tannin has been reported to cause developmental depression. Rambutan contains many compounds such as alkaloids, flavonoid, polyphenols, saponin, tannin and fatty acid. Soya saponin and hederagenin are an example of saponin that can be found in rambutan [11].

1.2. Extraction Technique of Saponin

1.2.1. Saponin Extraction

Saponin has been isolated from plants that are high in saponin used in the industry and from plant extracts. The initial step in the production of saponin includes the extraction of them from the sources of the plant. The main factors that determine process effectiveness are the extraction solvent, extraction conditions such as temperature, pH, and solvent-to-feed ratio, and the feed material properties such as composition and particle size. Different types of extraction methods can be used to extract material from plants. In general, it is possible to extract saponin extraction techniques into two categories which are traditional and green technologies. Traditional extraction techniques include maceration, Soxhlet, and reflux extraction; green technologies include ultrasound, microwave, and rapid solvent extraction [20-22]. The solubility of plant material solute in the solvent is a prerequisite for conventional extraction. As such, it frequently uses a lot of solvents to extract the desired solute, however heating and mechanical shaking or stirring might also help occasionally. Besides, environmentally friendly extraction methods included less harmless chemical synthesis, the use of safer chemicals, energy efficiency, the use of renewable feedstock, and pollution control. Ethanol and methanol were the extraction solvents used to extract saponin from plant material; ethanol was selected because of its greater environmental friendliness. Maceration extraction is sometimes assisted using a mechanical shaker to decrease the extraction period because the extraction process is laborious and might take weeks at a time when using this approach [20]. Subsequent extraction methods using a solvent such as chloroform and hexane are then carried out to remove lipids or fats that may interfere with subsequent analysis [6], also conducted a defatting process in their saponin extraction from the leaves of *Medicago arabica*, *Antonia ovata* and *Tribulus terrestris* leaves respectively.

1.2.2. Maceration

One separation technique that was quick and economical is the extraction of maceration The maceration extraction procedure, which is a solid-liquid extraction, involves soaking the plant material in a particular solution for a certain amount of time to extract the bioactive chemical (solute) within the material. Some of the main factors that determine the effectiveness of the maceration process are solubility and efficient diffusion. Solubility is controlled by the simple "like dissolves like" law, which states that polar compounds dissolve in polar solvents and nonpolar compounds dissolve in nonpolar solvents [22]. The rate at which a solvent dissolves in an extraction solvent is determined by measuring the mass transfer rate of the solvent from the plant material to the solvent [6]. The concentration gradient at the solid-liquid contact causes the solute to move throughout the plant material, suggesting that an effective diffusion is applied. No complex tools or equipment are required for the installation of a maceration extraction system, which has made it a popular choice for researchers. The sole critical aspect that must be considered in order to increase extractability is the awareness of the parallels between solvent polarity and bioactive component interest. The extraction process can take weeks, therefore it can be helpful to employ a mechanical shaker or magnetic stirring to speed it up. [9] found that maceration was a quick and efficient way to get rid of the phenolic compounds in chokeberry fruit. Under optimal conditions, chokeberry fruit with a solid-solvent ratio of 1:20, 50% ethanol, and a particle size of 0.75 mm yielded notable amounts of total phenols and total anthocyanins.

1.2.3. Liquid-Liquid Extraction

Another term for liquid-liquid extraction (LLE) is solvent extraction or partitioning. It is a process of separating components from liquid mixtures that involve the dilution of some or all of the initial mixture components and the usage of liquid solvents. Besides, the efficient separation of homogenous liquid solution constituents (solutes), LLE is a useful approach. In this separation step, a second liquid solvent that is either partly or immiscible with the feed is added, and the mixture's solutes are split between the two phases. The partition, also known as the coefficient of distribution, is a quantitative indicator of how a variable is distributed between the two phases. The distribution coefficient is the ratio of the solute concentrations in the two different solvents when equilibrium is reached by the system. If the distillation process is not possible, takes more energy, or is too complicated, LLE is used as a method of separation. This extraction is also used if the material is heat-sensitive and non-volatile [22].

1.3. Chromatogram Analysis of Saponin

1.3.1. Analytical HPLC

The measurement of saponin in plant material can be done using a variety of techniques. The number of saponin can be determined using spectrophotometry, which is a straightforward and useful technique. Many different saponin found in plant extracts have been effectively separated, purified, and determined using thin-layer chromatography (TLC). Additionally, the technology of high-performance liquid chromatography (HPLC) is frequently used for the quantitative measurement of saponin [12]. For the separation, identification, and purification of saponin, the normal-phase

and reverse-phase HPLCs are frequently utilized. This method is fast, targeted, and extremely sensitive. HPLC can alter the separation of saponin with a range of stationary and mobile phases. The majority of research focuses on triterpene saponin and often uses a C18 column with an aqueous acetonitrile mobile phase gradient, which are the chromatographic conditions recommended for HPLC ultraviolet (UV) analysis. The table below shows some plants using a different method for HPLC analysis of saponin. Each HPLC method's objective for analysis may change depending on the stage of development. For the quantitative and qualitative study of organic chemicals in unprocessed plant extracts, such as alkaloids from *Nicotiana* spp., HPLC is often used in phytochemical studies. A variety of HPLC techniques have been established in a distinct field for the quantification of substances such as sildenafil in human plasma, acetazolamide, furosemide, and phenytoin in suspensions [15].

1.3.2. Preparative HPLC

Preparative high-performance liquid chromatography is widely used for the extraction of components from difficult materials, such as biological and natural product samples. Since high-throughput purification is required for combinatorial chemistry and drug discovery, preparative HPLC systems have been developed and employed as a result of instrument technological developments. Currently, the main components of commercially available preparative HPLC systems include automated sampling, precise fraction tracking, and multi-channel parallel separation modules. These components may completely satisfy the requirements for separation repeatability and selectivity. Nowadays, preparative HPLC is one of the key methods for efficiently and with high purity isolating and purifying molecules of interest [21]. Due to its wide range of applications and superior separation power, the reverse phase mode is the most frequently used separation mode in both preparative and analytical HPLC. The hydrophobic interaction is primarily responsible for separations in Reverse Phase mode. The co-eluted chemicals, which share a comparable hydrophobicity, appear to be challenging to isolate and purify in Reverse Phase mode. To increase the separation selectivity of the co-eluted components, a substitute for RP-HPLC with a different retention mechanism must be developed [12].

1.3.3. Potential and Challenges of Saponin Development

Saponin contains so many different qualities, including the ability to froth and emulsify, research on saponin has gained a lot of attention. In addition, saponin has been utilised in food as a natural surfactant and preservative to prevent microbiological food spoilage [19]. It also possesses hemolytic, molluscicidal, anti-inflammatory, antifungal or anti-yeast, antibacterial or antimicrobial, antiparasitic, antitumor, and antiviral pharmaceutical qualities in addition to immunological adjuvant and

hemolytic activity. The pharmaceutical industry has also shown interest in saponins since some of them serve as the building blocks for the semi-synthesis of steroidal medicines [19]. With diverse pharmacological and surface-active properties, saponin have been profiled to have valuable activities. Nowadays in various industries such as food, medicine, and cosmetics, saponin has been commonly used. Saponin are compounds that present in a most of vegetables, beans, and herbs and come mainly from plants. However, because there is mounting proof that both food and plant-based saponin sources can lower cholesterol and even fight cancer, their importance has grown in recent years. Clinical experiments have shown that saponin, one of these health-promoting chemicals, affects the immune system in ways that help shield the body from cancer and even lower cholesterol. Saponin reduces blood lipids, decreases the risk of cancer, and decreases blood glucose response. Most of plants have triterpene saponin present and also show a broad range of pharmacological activities including vasoprotective, gastroprotective, expectorant, anti-inflammatory and antimicrobial properties. Recently, their cytotoxic, cytostatic, pro-apoptotic and anti-invasive effects have indicated the possible anticancer activity of saponin [18]. Natural phytochemicals called saponins have many useful capabilities in the development of chemopreventive and chemotherapeutic medications. However, their progress is hampered by several issues. Poor bioavailability resulting from low permeability and saponin hydrolysis by microflora is one of the main reasons limiting their pharmacological effects *in vivo*. Furthermore, issues including bitterness, cytotoxicity, and instability under specific circumstances must be resolved to effectively use saponins in food functions and applications. When taken in high amounts over an extended time, several saponin glycosides can have hazardous consequences that include excessive salivation, vomiting, diarrhea, lack of appetite, and even death. A noteworthy obstacle to be addressed is the emergence of saponins as inexpensive substitutes. Researchers are attempting to create immunostimulatory saponins by exploring various structural alterations to the skeleton of QS saponins, such as modifications to the sugar and aglycone compositions, and by developing new synthetic techniques.

1.3.4. Conclusions and Recommendations on Saponin

The important new information about the possible uses of saponin derived from *Nephelium lappaceum* (rambutan) leaves has been made possible by studies on its surface-active characteristics. Because of their surfactant qualities, saponins are well-known, and rambutan leaves are a great source of these substances. There are obstacles in the way of saponins' development as biological surfactants, though. To properly use saponins in a variety of applications, these obstacles include overcoming their bitterness, cytotoxicity, and instability under specific conditions.

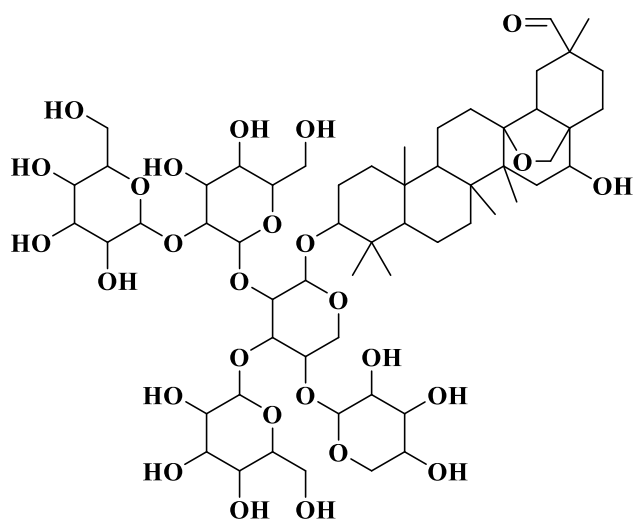


Figure1: Structure of Saponin [18]

Furthermore, there is a big obstacle that needs to be cleared with the introduction of saponins as inexpensive substitutes. In summary, more study is required to properly understand the difficulties and potential of rambutan leaf saponins, even though they show promise as biological surfactants. Future research should concentrate on addressing the drawbacks of saponins, such as their cytotoxicity and bitterness, and look at practical ways to extract and use them at a reasonable price. Furthermore, it would be advantageous for saponins to be used in a variety of industries if efforts were made to improve their stability in diverse environmental settings.

Conflict of Interest

The authors declare there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgment

The authors would like to thank Universiti Malaysia Pahang for funding with Postgraduate Research Grants Scheme (Vot. no: PGRS220375).

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