

Bioactive Compounds of Sanrego (*Lunasia Amara* Blanco.) Extracted Using Different Methods: A Review

Mawar Dhani Putri^{1*}, and Abu Bakar Tawali^{1,2}

¹Departement of Agricultural Technology, Faculty of Agricultural, Hasanuddin University, 90245, Makassar, Indonesia

²Center of Excellence in Science and Technology on Food Product Diversification, 90245, Makassar, Indonesia

Abstract. The use of plants as medicine has been trusted by the community since time immemorial. Sanrego (*Lunasia amara* Blanco.) is one plant that has been believed to be an aphrodisiac. Sanrego is a wild plant found in the forests of Sulawesi, Java, Borneo, Maluku, Philippines, Papua New Guinea and Australia, and belongs to the *Rutaceae Family*. Sanrego has several properties such as treating stomach pain, anti-inflammatory, anti-cancer, snake bites and increasing sexual aggressiveness. This is because sanrego has bioactive ingredients as pharmacological. Sanrego extract contains secondary metabolites or bioactive compounds that include flavanoids, tannins, alkaloids, steroids and saponins. Given the large variety of bioactive compounds in a sanrego plant, it is necessary to pay attention to extraction techniques in filtering these compounds. Some environmentally friendly extraction methods that can be used include maceration extraction methods, soxhlet, reflux, ultrasonic, microwave, and enzyme assistance. This review discusses and describes the various extraction techniques used to obtain plant bioactive compounds. These extraction techniques include conventional extraction techniques and non-conventional extraction techniques. This review analyzes the advantages and disadvantages of this extraction technique. This review covers the application of extraction methods to sanrego plants.

1 Introduction

Indonesia is a country in Southeast Asia that has biodiversity and richness. Medicinal plants are Indonesia's wealth that has been recognized by the world [1]. The French botanist Bontius once recorded this in a compilation of lists of Javanese medicinal plants. For centuries, various diseases have been treated using medicinal plants. According to a WHO report, traditional medicine is an important component of healthcare provision in many African countries [2]. Ethiopia still commonly practices traditional medicine, with about 90% of the country's population using plant-based traditional medicine as a primary service.

* Corresponding author: mawardhanip@gmail.com

Medicinal plants found in Indonesia are Sanrego (*Lunasia amara* Blanco.). Sanrego are wild plant that can be found in the forests of Sulawesi, Jawa, Kalimantan, Maluku, the Philippines, Papua New Guinea and Australia. Sanrego belongs to Rutaceae family [3]. This plant has several properties such as aphrodisiacs [4], anti-inflammatory [5, 6], anti-malarial [7] anti-diabetic [8]. The pharmacological activity produced by sanrego extract is due to the content of secondary metabolites in the form of coumarins, alkaloids and flavonoids [8]. These secondary metabolites or bioactive compounds are what make sanrego medicinal.

Phytochemicals in plant life are divided into two groups [9]. Primary metabolites constitute the first group, needed by plants for growth and development. Substances needed for growth and development include nucleic acids, carbohydrates, fatty acids, proteins and molecules present in plants, such as growth regulators and cell wall components. Secondary metabolites are the second group that plants use to enhance their ability to survive. In plant physiology and biochemistry, secondary metabolites have various functions such as insecticidal, fungicidal, antibacterial or antiviral properties [10]. Therefore, these secondary metabolites can be considered bioactive compounds because it exerts pharmacological or toxicological effects on animals and humans [9].

Given the many variations of bioactive compounds in a plant, it is necessary to pay attention to techniques in filtering these compounds. Many efforts have been made by researchers to find better extraction methods to obtain compounds with high efficiency and efficacy. Efficiency refers to the result of extraction, while efficacy refers to the potential effect produced [11]. There are several extraction methods to obtain plant bioactive compounds. Extraction methods can be divided into two groups namely conventional methods and non-conventional methods. Non-conventional extraction methods are known to be more environmentally friendly due to the use of fewer synthetic and organic chemicals, short time and better quality extracts [12].

The conventional method is also still considered as a reference method for comparing the success of newly developed methodologies. But what is an obstacle, conventional methods do not filter bioactive from plants [12]. To our knowledge, many previous bibliometric studies have focused on the sanrego plant, such as the bioactive compounds it contains [13] [8, 3], extraction methods and solvents used [4, 14], and the spread of sanrego plants [15]. However, there are no studies that discuss the comparison of various extraction methods in filtering the bioactive compounds of sanrego plants. This review aims to provide a comprehensive overview of sanrego plants, their distribution and extraction methods.

2 Sanrego (*Lunasia amara* Blanco) description

Sanrego is a plant in the Rutaceae family that has only one dioecious species (*Lunasia amara* Blanco.) and two varieties namely *L.amaravar.amara* and *L.amaravar.babuyanica* [16]. This variety is also known as a tree ('lunas-kahoy') and a vine ('lunas-bagon') [17]. Both thrive at moderate altitudes and sprout in soil habitats such as clay. This tree sprout in habitats with dry shrubs and replanted farm. In contrast, vines are located near rivers with dry shrubs in Midland [17].



Fig. 1. Sanrego (*Lunasia amara* Blanco) [17].

Generally, the variety of sanrego used locally is the tree type. The sanrego plant is a shrub with a height of up to 12 meters and a diameter of up to 15 centimeters, rarely branched, and all parts of the plant are covered with scales. Single leaves, stacked at the ends of the branch, inverted lanceolate to inverted ovate with a length of 5.5-60 centimeters, the base pointed to slightly rounded or heart-like, with scattered oil spots. The flowers are panicles, male panicles and female panicles, greenish yellow to white in color. The fruit consists of 1-3 chambers, one seed. Seeds are inverted ovoid, dark brown to reddish brown in color, with thick cotyledons containing oil [18]. There are three yellowish fruits and a length of 1 centimeter or more, clearly marked with ribs, and open along the upper vein and suture [3].

Sanrego is known by several names in the Philippines such as apdongkaho, lunas, lunas-bondok, saltiki, santiki, labau, pait (Tagalog), lubi-lubi (Central Bisaya), labau (South Bisaya Luzon), marmango (Ibanag), dayandang, paitan (Ilokano), pait, papait, bungalay (Bikol), lunan (Pampango), palatangan (Gadang). Indonesia also has several names in each region, for example sanrego, beta-beta (Sulawesi), kernaitan, maitan (Java), kamaitan (Madura), pintan, makelumhalaluna (Minahasa) [18].

The utilization of the sanrego plant as a medicine because it contains bioactive compounds or secondary metabolites such as flavanoids, alkaloids, tannins and other compounds [14], steroids and saponins [13, 19]. Indonesian people, especially in South Sulawesi, utilize the sanrego plant as an aphrodisiac, which is a food or medicine that arouses sexual desire [20]. The use of sanrego as an aphrodisiac stems from information developed in the community regarding a horse that licked the sanrego wood, ate the leaves and bark, thus gaining an increase in libido with sexual power [4]. The sanrego plant has also been widely used in Indonesia as a remedy for swollen limbs, skin diseases, and inflamed or irritated eyes, while the wood decoction as an aphrodisiac [21]. The spread of sanrego has been widespread in Indonesia and is found in various regions on the island of Sulawesi to the western island of Papua [15].

3 Bioactive compounds of sanrego (*Lunasia amara* Blanco)

Plants can produce compounds that have pharmacological effects on humans, namely bioactive compounds. These plants produce bioactive compounds as secondary metabolites [22]. Secondary metabolites are metabolites produced in the post-growth stage (although they have a survival function) [12]. In different species, secondary metabolites are produced through the evaluation and species needs. For instance, the scent synthesis from flower species to lure insects for pollinations and conception, the chemicals synthesis have evaluated to be to suppress the growth of surrounding plants [23].

Research on bioactive compounds in sanrego plants has been carried out, including alkaloid compounds as aphrodisiacs [4], alkaloid derivative is lunacridine as an anti-cancer agent [14], sanrego also contains steroid, phenolic, saponin, alkaloid and coumarin compounds as antioxidants [13]. The usefulness of sanrego as an aphrodisiac is supported by

research conducted by Muchsin Darise, a pharmaceutical researcher from Hasanuddin University, South Sulawesi. Sanrego has been reported to be a good sexual stimulator [3]. Based on the results of qualitative phytochemical tests (Thin-Layer Chromatography method) conducted at the Pharmacognosy Phytochemistry Laboratory, Faculty of Pharmacy, Hasanuddin University, it is known that sanrego wood extract contains 5 (five) secondary metabolite compounds consisting of flavanoids, tannins, alkaloids, steroids and saponins. However, compounds that have positive content in sanrego wood extract are flavanoids, alkaloids and steroids which are characterized by a more intense color change than other compounds [19].

3.1 Flavonoid

Flavonoids are a group of natural substances that are included in the group of secondary plant substances that have a polyphenol structure [24]. Flavonoids contain *phenylchromen-4-one* which can be substituted with a phenyl ring C2 or C3 to produce flavone and isoflavone structures. Furthermore, C4 (ketone group), C2-C3 (saturated or olefin) plus hydroxy or methoxy on phenylchromen-4-one and phenyl ring results in the formation of flavanols, flavanones, flavonols, isoflavones, flavones and anthocyanidins [25] (Figure 2).

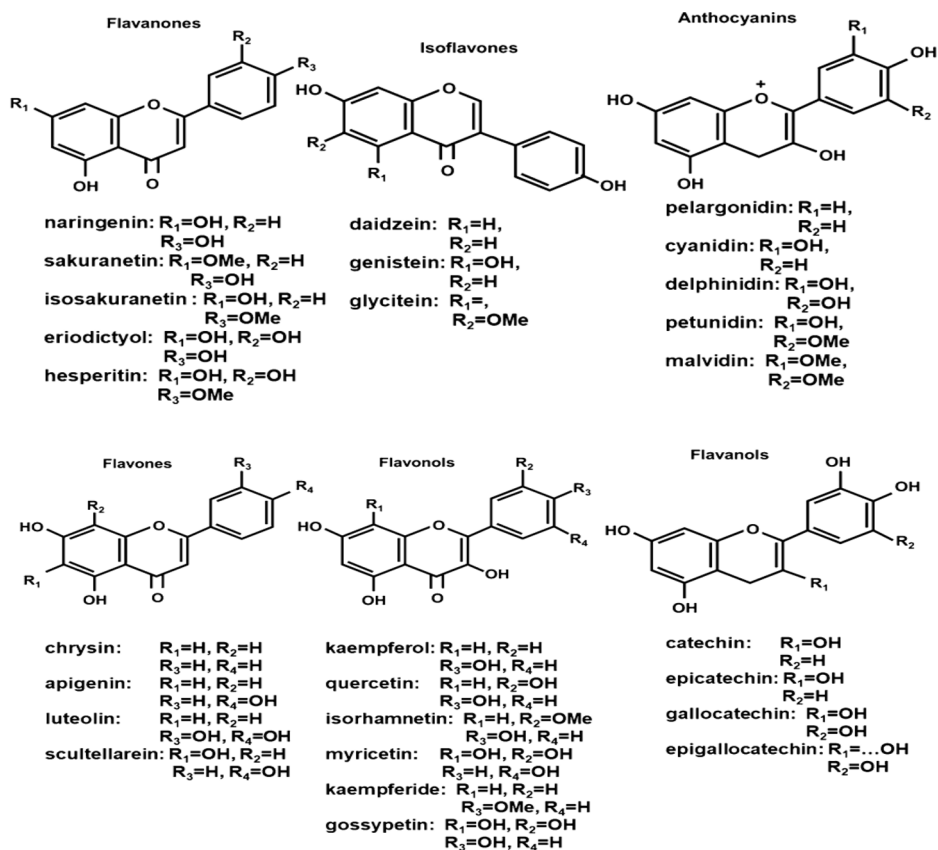


Fig. 2. Structure of favonoids and some individual members of each class [25].

Flavonoids are used by vegetables for their growth and defense against diseases [26]. The biological and oxidative properties of flavonoids are responsible for their antidiabetic,

antiallergic, antiinflammatory, cardioprotective, antioxidant, and free radical-fighting effects [27]. Flavonoids considered thought exert positive effects on human health, their current attention is for disease therapy and chemoprevention. One important mechanism underlying the actions of flavonoids and polyphenols in foods is related to the suppression of downstream reactions such as oxidative stress and inflammatory diseases [25]. Flavonoid capture free radicals and subsequent damage by forming relatively stable phenoxy radicals [28].

3.2 Alkaloids

Alkaloids are nitrogen-containing heterocyclic compounds, usually with strong activity and a bitter taste [22]. Alkaloids in sanrege wood have the empirical formula $C_{17}H_{23}NO_4$ and contain two methoxyl groups, one N-methyl group and two active hydrogen atoms and are quinoline alkaloids which have two carbon rings with one nitrogen atom [14]. Several phytochemical studies in *Lunasia amara* have identified five main groups of quinoline alkaloids based on their biogenic origin [3].

3-Dimethylallyl-2-Quinolones is a broad and well-studied group of alkaloids, produced biogenetically through the pre-condensation of anthranilic and acetic acids to produce quinolones, which are highly oxidized quinoline derivatives. In *Lunasia amara*, six classes belonging to this group were identified, namely lunacridine and hydroxyl derivatives [29], lunidine [3], and hydroxy derivatives [29], as well as lunidonine. Other unidentified alkaloids that belong to this group are lunolone [29] (Figure 3).

Furoquinolin is chemically a simple type of nitrogen metabolite. Most of them have aryl groups, diverse based on the method of ring substitution in the benzenoid structure and the presence of methoxy groups in C-4 [30] [3]. Two inactive quinoline alkaloids identified in this type of *Lunasia amara* are kokusagine (7,8-methylenedioxydictamnine) and skimmianine [29] [3] (Figure 4).

Furoquinolon is a group of alkaline compounds known as artifactual natural elements. Based on their basic ring structure either as furoquinoline or as 2-isopropyl-2,3-dihydrofuroquinoline [3]. The major isopropyl-bearing alkaloid metabolite isolated from *Lunasia amara* is lunacrine along with small amounts of lunine [29] [3]. The two additional alkaloids identified that have hydroxyl groups on the isopropyl moiety are hydroxylunacrine and hydroxylunine [29]. Quaternary furoquinoline alkaloids have been identified in *Lunasia amara* to be the fifth member [3] (Figure 5).

2-Arylquinolines and 4-quinolones are a alkaloids category that are biogenetically derived through the condensation of anthranilic acid and other aromatic acids such as phenylalanine and tyrosine [3]. 2-Arylquinolines identified from *Lunasia amara* are 4-methoxy-2-phenylquinoline [33] and 4-methoxy-2-(3',4'-methylenedioxyphenyl) quinoline (graveoliline) [29] [3]. While 4-quinolones in *Lunasia amara* are known to contain 2-phenyl-4-quinolones and lunamarine [3] (Figure 6).

Sesquiterpenes are a large family of C-15 isoprenoids found in higher and lower plants, microbes and some marine organisms. Sesquiterpenes are key components of many essential oils, which are commercially important to the flavor and fragrance industries. The leaves of *Lunasia amara* produce an essential oil consisting of I-elemene, germacrene, bicyclgermacrene, bicycloelemene, I-bournene, I-elemene, I-farnesene and I-cadinene [35][3] (Figure 7).

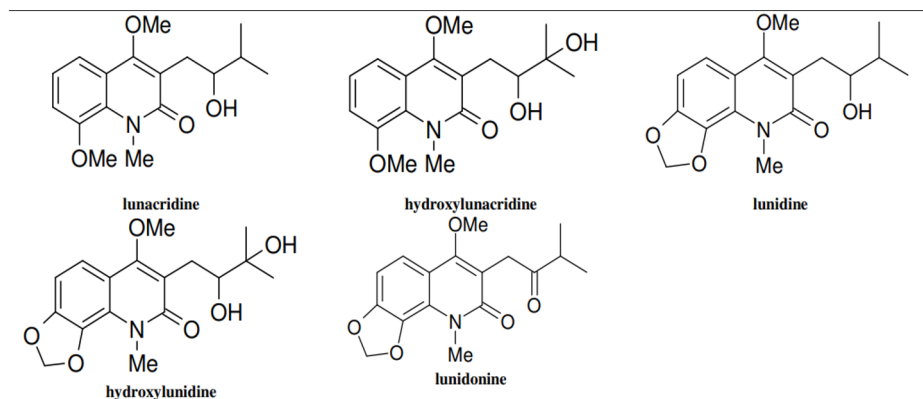


Fig. 3. 2-Quinolones of *lunasia amara*.

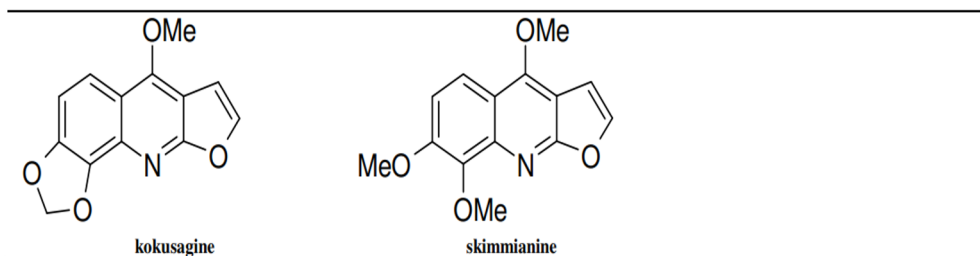


Fig. 4. Furoquinolines of *lunasia amara*.

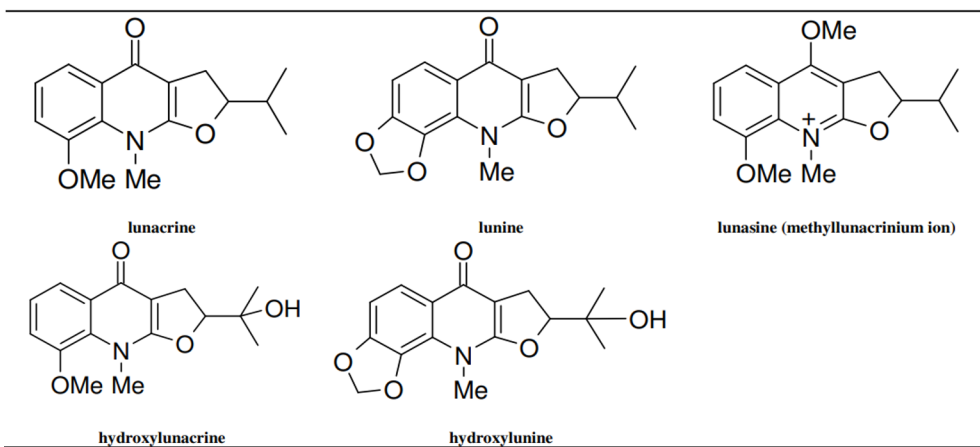


Fig. 5. Furoquinolones of *lunasia amara*.

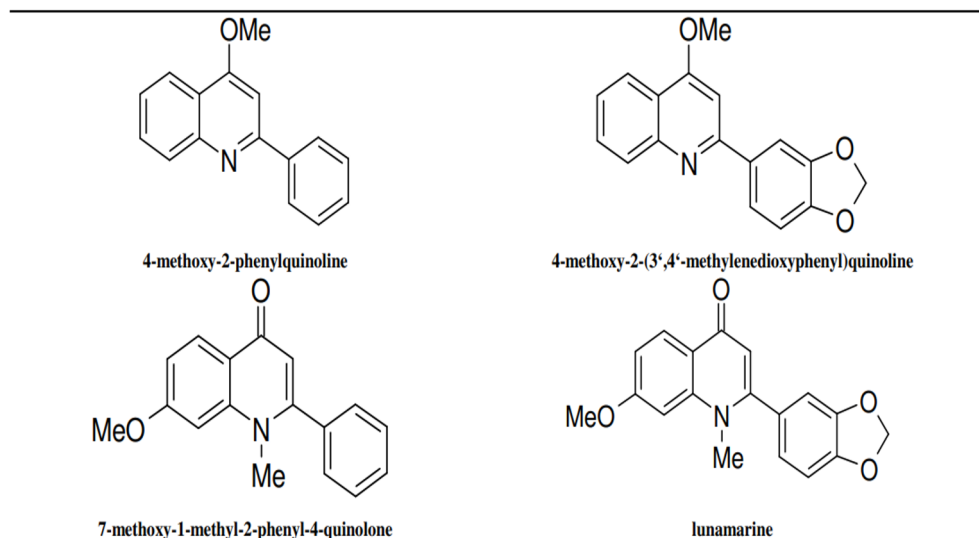


Fig. 6. 2-Aryquinolines and 4-quinolones of *lunasia amara*.

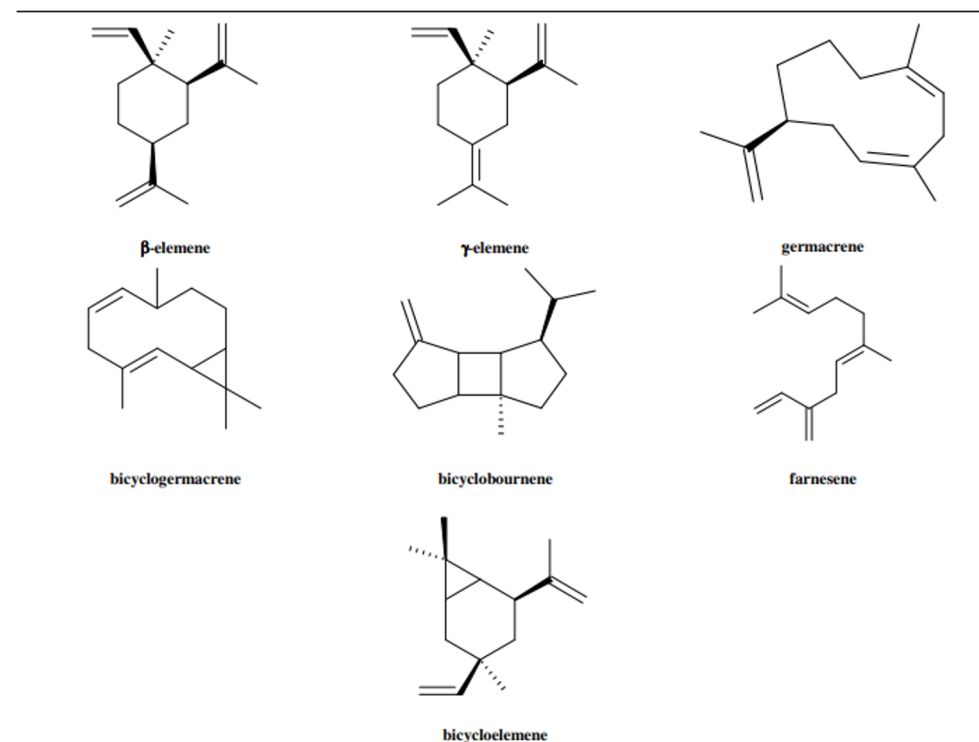


Fig. 7. Sesquiterpenes in the essential oil of *lunasia amara*.

3.3 Steroids

Steroid compounds can be found in animals and plants, having similar functions in living organisms. Steroids are one class of secondary metabolite compounds that have bioinsecticidal, antibacterial, antifungal and antidiabetic activities [36]. In plants, steroids are divided into two categories: phytosterols and brassinosteroids [37]. A group of steroid

alcohols found in plants, known as phytosterols are naturally beneficial and can reduce cholesterol levels by reaches 15% for humans [38] [37], and act on cancer prevention [37]. The effects of brassinosteroids (BRS) include various responses such as cell elongation and division, deoxygenation inhibition, and genes that regulate stress. BRS tend to counter biotic and abiotic stresses from plants [39] [37].

4 Sanrego (*Lunasia amara* Blanco) extraction

Given the large variety of bioactive compounds in sanrego, The extraction technique used needs to be considered so that it can filter compounds that bring health benefits. To conduct futher separation, identification and characterization of bioactive compounds followed by an appropriate extraction process [12]. Extraction involves separating the active parts of the plant as drugs using selectivity and standards [40]. Here are some stages of natural product extraction: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvent; (3) the solute diffuses out of the solid matrix; (4) the extracted solute is collected [41]. [42] in his review explains that there are several factors to consider in the extraction process, including the nature of the solvent to be used, particle size of the raw material, solvent ratio, extraction temperature and duration.

The choice of solvent is very important in the extraction process, this will affect the compounds that will be separated from a plant. This solvent selection can be seen from the polarity of the desired solute [43]. Solvents with polarity values close to the solute's polarity tend to perform better, based on the laws of similarity and intermiscibility. In addition, particle size of material to be extracted also needs to be considered. Finer particles equate to better extraction efficiency. Small particle size increases extraction efficiency by increasing solvent penetration and solute diffusion [44]. Even though they are small in size, too much dissolved substances in the solid will be absorbed and require extensive filtration for further processing.

Temperature in extraction process also needs to be considered because high temperature can increase solvability and diffusion [45]. High a temperature can lead to loss of solvent and can vaporize the compounds to be filtered. Once the balance of solutes inside and outside the solid material is achieved, increasing the time will not affect the extraction. The extraction results are directly proportional to the ratio of solvent to solid. Excessive solvent extraction and long concentration times will be a consequence of too high a solvent to solids ratio [42]. There are two types of extraction methods: convensional and modern. The Soxhlet method, maceration method and reflux method are the three most commonly used conventional methods [46]. But this conventional method has drawbacks such as long extraction time, more in solvents use and high purity, lower extraction selectivity and thermal decomposition of compounds that cannot withstand heat at higher temperatures [47]. Modern or unconventional extraction methods include ultasound-assisted extraction (UAE) [48], pulsed-electric field extraction (PEF) [12], enzyme-assisted extraction (EAE), microwave assisted extraction (MAE) [49], and supercritical fluid extraction (SFE) [11]. Extraction of bioactive compounds in sanrego (*Lunasia amara* Blanco) uses more conventional methods, this is because in addition to easy workmanship and also cheaper than modern or non-conventional extraction techniques.

4.1 Conventional Extraction Techniques

4.1.1 Maceration extraction

Maceration extraction method is an extraction method that does not involve heating, but involves the polarity of the solvent to attract active compounds. The mechanism of action of

this method is by soaking plant material (coarse or powder) using a suitable solvent and standing at room temperature for at least three days and occasionally stirring to attract the active compounds [50]. This processing is intended to soften and destroy plant cell walls [51]. The filtrate and residue were then separated and the resulting residue was macerated again with the solvent using the same process. This process is carried out continuously until the color of the resulting filtrate is constant. Collect the filtrate and evaporate using a rotary evaporator until a thick extract is formed [50].

The disadvantage of maceration extraction method is that it requires a long time and low extract efficiency [9]. In addition, organic waste becomes a problem due to the use of large amounts of solvents [51]. However, this method is still widely used because it is simple without heating, making it suitable for compounds that are not resistant to heat [52].

4.1.2 Soxhlet extraction

Soxhlet extraction method is a traditional technique that is widely used using hot solvents. One of the main advantages of this method are compounds with average to low solubility can be extracted by this method [53]. In 1879, the Soxhlet method was first used to extract lipids, but since then it has developed into a widely used and efficient method for extracting many natural plant bioactive compounds [54]. The principle of soxhlet extraction is repeated filtration so that the results obtained are perfect and the solvent used is relatively small [55].

This method requires a smaller amount of solvent compared to the maceration method [51]. However, the soxhlet extraction method has several disadvantages, among others, it cannot be used on materials that have a hard texture [56]. Other drawbacks are exposure to harmful and flammable liquid organic solvents, as well as potential toxic emissions during extraction [51]. Compounds extracted using this method tend to decompose or degrade during heating [9].

4.1.3 Reflux extraction

Reflux extraction is an extraction that uses solvent-based heating for a certain amount of time with a limited and relatively constant amount of solvent [57]. In the reflux method, the solvent used evaporates at a high temperature, but when the condenser cools it, all remaining solvent condensed in the condensation process is returned to its original state and remains in place during the reaction. Next, the solution is filtered using a filter cloth. The filtrate is evaporated using a rotary evaporator [58]. This method is generally used to synthesize volatile compounds [59].

The advantages of the reflux method over other conventional methods are shorter extraction time and less solvent cost due to solvent reuse. However, the reflux method has the disadvantage that phenolic compounds or compounds that are not heat resistant may be contaminated during evaporation or decomposed at high temperatures [60].

4.2 Non-conventional extraction techniques

4.2.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound is an electromagnetic wave with a frequency higher than the sound waves heard in the human ear. The range ultrasound utilized is from 20kHz to 2000kHz [9]. Natural compounds will be released from plant tissues through high-frequency sounds, thus damaging cell walls [40]. Ultrasound in the solvent producing cavitation accelerates the dissolution and diffusion of the solute as well as the heat transfer, which improves the extraction efficiency [42]. This method is a simple method and requires little cost and can be

used on a small or large scale [46]. Compared to conventional methods, this method requires less solvent and shorter extraction time. However the use of ultrasonics in the food industry is usually optimized due to cost considerations [60].

4.2.2 Pulsed-Electric Field Extraction (PEF)

PEF is a non-thermal extraction method that applies short, high voltage pulses to destroy membrane structures and release the content which increases the extraction yield [60]. Pulsed electric field extraction significantly increases the extraction yield and decreased the extraction time because it can increase mass transfer during extraction by destroying membrane structures [42]. The effectiveness of pulsed-electric field treatment depends on several parameters including field strength, specific energy input, pulse number and treatment temperature.

4.2.3 Enzyme-Assisted Extraction (EAE)

This extraction method is a method that utilizes the enzyme's ability to break cell wall compartments [46]. The extraction rate can be increased by hydrolyzing membrane components in cells that impair selective permeability and releasing compounds with the help of enzymes. The structure of the cell membrane is mainly formed by macromolecules of polysaccharides and proteins, and at high temperatures the proteins will undergo denaturation which will affect the extraction efficiency [60]. EAE is used in conjunction with other extraction techniques because enzymes make phytochemicals that cannot be extracted accessible to solvents [9].

4.2.4 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction, also known as microwave extraction, is a technique of extracting natural products using microwaves to heat solutes with and solvent. The heat generated facilitates the diffusivity of the solvent into the sample to improve the phytochemical diffusion of the target out of the sample [61]. Microwave frequencies range from 300MHz to 300GHz [9]. Compared to conventional extraction, selective migration of compounds can be transferred from material to solvent in a shorter time with local temperatures and pressures generated during extraction [60]. Although the time required is shorter, the energy costs during this extraction process may be higher.

4.2.5 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction is an extraction method that uses supercritical fluid as a solvent for extraction. Supercritical liquids are ideal solvents that have solubility similar to liquids while their diffusivity is similar to gases so that they are able to dissolve various kinds of natural materials [60]. One of the commonly used SFs in this extraction method is supercritical carbon dioxide (S-CO₂). The application of S-CO₂ in this method has a great advantage because it is a mobile phase that is non-toxic, cheap, non-flammable so it does not cause disposal problems. The main drawback of S-CO₂ is its low polarity [62]. As a low-polarity solvent, carbon dioxide is more suitable for the extraction of nonpolar compounds. However, its application can be extended to other groups of compounds by adding small amounts of polar organic solvents to the extraction process to allow extraction of polar compounds [61]. Among the organic solvents suitable for use as solvents along with carbon dioxide are ethanol and ethyl lactate [63].

The sanrego (*Lunasia amara* Blanco) extraction process is carried out in several stages. The first stage is that the parts of the sanrego plant (leaves, wood, roots and bark) to be extracted are washed thoroughly using running water. Sanrego is dried in the sun to remove the water content. This drying also aims to minimize microbial growth on the sanrego to be extracted. Then the size is reduced to facilitate the extraction process. Sanrego parts that have become powder or powder are extracted using appropriate methods and solvents to obtain thick sanrego extract.

5 Bioactive compounds of sanrego (*Lunasia amara* Blanco) extracted using different methods

Sanrego plant samples that have become thick extracts are then tested to determine the bioactive compounds contained in them. Testing of bioactive compounds of sanrego compounds can be done by phytochemical profile tests using several chemical reagents, Thin-Layer Chromatography and GC-MS (Gas Chromatography-Mass Spectrometry). Here are some bioactive compounds from sanrego plants extracted using several methods.

Table 1. Bioactive compounds of sanrego (*lunasia amara* blanco) extracted using different methods.

Plant parts	Methods	Solvents	Bioactive Compounds	Results	References
Wood	Maceration	Ethanol 70%	Antioxidants	IC ₅₀ = 69,46 µg/ml (0,069 mg/ml)	[13]
			Steroids	+ red ring	
			Phenolics	+ dark purple	
			Alkaloids	+ brown sediment	
			Coumarins	+ blue fluorescence	
			Saponins	+ stable foam	
Wood	Maceration	Ethyl acetate	Scopoletin	Target protein is CA9	[8]
			umbelliferone		
Bark	Maceration	N-hexane	+ steroids	-	[4]
		Chloroform	+ steroids + flavonoids	-	
		Ethyl acetate	++ steroids + flavonoids	-	
		Metanol	+ alkaloids + steroids + flavonoids	-	
Wood	Reflux	Methanol	Lunacridine	37,52 µg/µL	[14]
	Maceration			35,03 µg/µL	
Wood Leaf	boiling	Water	Alkaloid quinoline:	-	[3] [31] [33] [34] [29]
1. 3-Dimethylallyl-2-quinolones					
2. Furoquinolines					
3. Furoquinolones					
4. Arylquinolines					
5. 4-quinolones					
6. Sesquiterpenes					

Wood	Boiling	Water	11 regulated sperm proteins (Enol, PGK2, F1-ATP synthase, FHAL-ATP synthase, LDHC, GLUL1, ODF2, Sp17, asparaginase, DNAJB12 and tubulin)	-	[64]
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6 Conclusion

The sanrego plant has the potential to be used as a medicine. This is because of the many bioactive compounds that are pharmacological in nature. Bioactive compound contained from sanrego plants such as alkaloids, flavonoids, steroids and the form of proteins used as anticancer targets and sperm proteins. The large variety of bioactive compounds of sanrego plants requires attention to the screening technique, namely extraction techniques that are in accordance with the type and group of bioactive compounds. The method used must also be appropriate, because there are groups of bioactive compounds that are not resistant to high temperatures. In the extraction process, the choice of solvent is very important, this will affect the compounds to be separated from a plant. This solvent selection can be seen from the polarity of the desired solute.

7 Limitations and Challenges

This review has limited references regarding bioactive compounds in sanrego plants. Some of the studies or references used are not recent and some studies only focus on maceration extraction methods. The challenge in this review is the dosage of sanrego plant extracts used for animals and humans.

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