The Potential of Roselle as Health Supplement: Extraction, Phytochemicals and Future Perspective

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Abstract. Roselle (\textit{Hibiscus sabdariffa}) is a native plant in Malaysia and it is often reported on its nutritional and medicinal values. To date, numerous studies have been conducted on roselle, both calyx and seed, and it is proven that the major bioactive compounds in calyx are ascorbic acid and anthocyanin, whereas the seed is rich in carbohydrates, proteins and unsaturated fatty acids, which are a good source of minerals and antioxidants. The utilization of roselle is commonly noticeable in food, cosmetic and personal care industries. It is interesting to note that the constituents present in roselle calyx and seed extract, which are anthocyanin, fatty acids and tocopherol, make it possible to be exploited in the pharmaceutical industry. Thus, this paper discusses the potential of roselle extract to be used as a health supplement, by reviewing its extraction methods, selected phytochemicals and also its future perspective in the pharmaceutical industry.

1. Introduction

\textit{Hibiscus sabdariffa} which is also known as Roselle belongs to the family Malvaceae is native to Asia (India or Malaysia) or Tropical Africa. In Malaysia, it is usually called as asam susur or Ribena Malaysia. Roselle is a cold-sensitive plant, annual with smooth, cylindrical red stems that can grow up to 2.5m high. It is plants which have a deep penetrating taproot, typically dark green to red stems, with high minerals and polysaccharides contain green leaves, red calyces with 5 big sepals with a collar (epicalyx) of slim and pointed bracts around the base (Figure 1). The calyces enlarge and fully enclose...
the fruit, 5-valved green velvety capsule during immature with 3-4 seeds in each valve (Figure 2). The matured seeds are in brown, kidney-shaped in 3-5mm long (Figure 3). The plant takes only three to four months to reach the commercial stage of maturity where the calyces are harvested.

![Figure 1](image1.jpg)  
**Figure 1.** The Roselle plant with dark red stems, green leaves and red calyces.

![Figure 2](image2.jpg)  
**Figure 2.** (a) Red calyces fully enclose the (b) velvety capsule with immature seeds.

![Figure 3](image3.jpg)  
**Figure 3.** Matured Roselle seeds.

Roselle is one of the commercial crops that are mostly exploited for the food processing industry in Malaysia. However, the increasing of health awareness on processed food [1] generally have reduced the demand for roselle. The demand of roselle from the industry has been decreased drastically since a few years ago, hence the matured roselle was wasted. This can be seen from the annual report of planted area and production of industrial crops which reported by Malaysia Agricultural Department in the industrial plantation statistic. Table 1 showing the production of roselle in 2017 dropped almost half compared to the year 2015. There is no decrement in planted and harvested areas but the production reduced which means the industries demand is much lesser and the plantation waste is
increasing. Explore the potential of roselle plant in various industries is one of the ways of helping the farmer to harvest all the matured roselle and to reduce the plantation waste.

| Year | Planted area (ha) | Harvested area (Ha) | Production (Tonnes) |
|------|-------------------|---------------------|---------------------|
| 2015 | 83.2              | 69.8                | 411.0               |
| 2016 | 59.3              | 53.0                | 276.4               |
| 2017 | 58.6              | 53.8                | 183.0               |

Roselle calyces are edible. In Malaysia, roselle calyces are the most useful part. They are collected mostly for processed food such as jam, jellies, sauces and concentrated juice. The attractive deep red pigments from the calyces’ extract have been widely used in the food industry as a natural food colorant. In Egypt, calyces are used to make a slightly sour and refreshing drink well known as karkade [4]. In China, roselle calyces can be traditional medicine to treat hypertension, fever, cancer, improve the digestive system and leukaemia due to its high content of protocatechuic acid [5]. Studies showed the roselle calyces extract is rich in phenolic compounds and ascorbic acid which are known for great antioxidant activities with scavenging free radicals, 86% at the dose of 250μg [6]. Recent scientific research works have established the protective effect of the dried calyces extract in cancer prevention, anti-inflammatory activity, anti-mutagenic activity, tissue repairs and regenerate activity in wound healing [7-9].

In past decades, the scientific studies on roselle are mostly focused on calyces while the data on other parts of the roselle plant are very few. Recent studies shifted the focal point to roselle seed which been found in the high potential of seed oil extraction for food, personal care and industrial purposes due to the high content of fatty acid especially polyunsaturated fatty acid (PUFA), protein, Vitamin E and dietary fibre. However, roselle seed is only for plantation or throw as waste after the roselle calyces exploited. The plantation of roselle includes the area of Africa, Sudan, Taiwan, Myanmar, Thailand, Vietnam, China and so forth, but the utilization of seed is not popular yet. Some reported studies showed in some parts of Africa, the roselle seed type is bitter, are ground into a powder and added into the meal. Some of them extracted the oil from seeds for cooking purpose, roasted seed becomes a substitute for coffee. In Northern Nigeria, roselle seeds are fermented in the presence of some spices to prepare a food known as Mungza Ntusa [10].

This paper reviews the extraction methods usually used by the researchers to extract roselle calyces and seeds, its important phytochemical contents and the benefits, also will discuss on the future perspective of roselle potential in the pharmaceutical industry.

2. Extraction of roselle

Various extraction methods have been employed to extract both roselle parts, calyx and seed. The choice of extraction method is important for the highest yield separation and the highest purity isolation of the targeted compounds from the plant. Solvent extraction and distillation are widely used for active compounds extraction from plants, while maceration, hydrodistillation and Soxhlet extraction are the traditional techniques to extract seed, leaf, gall and stem [11-13]. Few factors are influencing the extraction yield and compound to be extracted. Solvents toxicity, compound degradation, process selectivity, plant parts used as starting material, extraction time, temperature, nature of the solvent, solvent concentration and polarity play important roles in the determination of quantity and secondary metabolites component of the extract [14]. The high solvent to sample ratio may decrease the yield due to too more time taken for the solvent to vaporise and contacted with the samples in thimble [15].

2.1. Calyx

Table 2 demonstrates several extraction methods that have been used by researchers to obtain roselle calyces extract. Generally, polar solvents are used to extract the calyces since the dominant compound
lies in the anthocyanin group, which is polar. Two major compounds are delphinidin 3-sambubioside and cyanidin 3-sambubioside, whereas delphinidin 3-glucoside and cyanidin 3-glucoside reported as minor compounds in the calyces’ extract. Besides these compounds, organic acids such as ascorbic, citric, malic, tartaric and polyphenolic acids are also reported in the extract [16]. According to a study conducted by Tsai et al. [17], 85% of the anthocyanin content in the calyx is delphinidin 3-sambubioside which is the major contributor to the antioxidant properties in the extract.

Table 2. Extraction methods used for extraction of roselle calyces

| Extraction method | Parameter | Yield | Compounds |
|-------------------|-----------|-------|-----------|
| SC-CO₂ (Modifier: 70% Ethanol) | P: 8-12 MPa T: 50 - 70°C F_modifier: 5 – 10% | 26.73% | Red colour (anthocyanin) |
| Ultrasonic-Assisted (Water (H₂O), ethanol (EtOH), hexane (Hex), dichloromethane (DCM)) | NA | H₂O: 11.52% EtOH: 1.48% Hex: 0.28% DCM: 0.28% | Polar compounds Anthocyanin |
| Ultrasonic-Assisted (80% EtOH) | Power: 180 W Frequency: 40 KHz T: 32°C Time: 120 min | 28.44% | Anthocyanin (1.763 mg Cya3G/g dw) Proanthocyanidin (0.745 mg CE/g dw) |
| Solid-Liquid (80% EtOH) | Rotation: 180 rpm T: 32°C Time: 120 min | 33.0% | Anthocyanin (0.902 mg Cya3G/g dw) Proanthocyanidin (0.188 mg CE/g dw) |
| Maceration (EtOH acidified with 1.5 N/L HCl) | Ratio: 85:15 T: 4°C | NA | Total anthocyanin content (1386 mg Cya3G/100g) Ascorbic acid (140.13 mg/100g) |
| Heat Assisted (H₂O) | T: 30°C Time: 30 min | 44.85% | Delphinidin-3-O-sambubioside (8.55 mg/g) Cyanidin-3-O-sambubioside (2.26 mg/g) Total anthocyanin (10.60 mg/g) |
| Ultrasound Assisted (EtOH:H₂O) | Power: 386.3 W Time: 42.9 min Ratio: 46.1 | 61.21 % | Delphinidin-3-O-sambubioside (16.17 mg/g) Cyanidin-3-O-sambubioside (7.38 mg/g) Total anthocyanin (23.08 mg/g) |
| Solvent (H₂O) | T: 100°C Time: 10 min Volume: 10 mL | NA | Total anthocyanin content (58.60 mg/g) Delphinidin 3-sambubioside (4.11 mg/g) Delphinidin 3-glucoside (0.15 mg/g) Cyanidin 3-sambubioside (3.81 mg/g) Cyanidin 3-glucoside (0.46 mg/g) |
| Water bath | T: 55°C Time: 2 hours | 28.3% | Delphinidin-3-O-sambubioside (56.5 mg/g) Cyanidin-3-O-sambubioside (20.8 mg/g) Quercetin (3.2 mg/g) Rutin (2.1 mg/g) Chlorogenic acid (2.7 mg/g) |

SC-CO₂: Supercritical carbon dioxide; P: Pressure; T: Temperature; F: Flow rate; Cya3G: Cyanidin-3-glucoside; CE: Catechin

2.2. Seed

Table 3 shows the extraction methods commonly used for extracting roselle seeds, which are the part that usually discarded after mature calyces are harvested. The seeds are rich in fats content, thus most
of the extraction techniques did not use solvent to help in the process, or used non-polar solvents such as petroleum ether and hexane. They have been reported high in nutritional value, especially with regards to the oleic-linoleic group of fatty acids and the tocopherols content. Several studies have found the essential polyunsaturated fatty acids in extracted roselle seed oil, where a majority of them showed linoleic acid ranked as the highest content of fatty acids of roselle oil that could be a good source of essential fatty acids. Soheir et al. [25] and Mohamed et al. [26] demonstrated higher linoleic acid contained in roselle seed oil; 38.46% and 40.1%, respectively. Meanwhile, linolenic acid also included in the extracted roselle oil, but only in lower percentage [26,27]. Besides, phytosterols group also detected in the oilseed.

| Extraction method          | Parameter                     | Yield         | Compounds                                  | Ref   |
|----------------------------|-------------------------------|---------------|--------------------------------------------|-------|
| SC-CO₂                     | P: 27.8 MPa                   | 12.6%         | α-Tocopherol (89 ppm)                       | Our data |
|                            | T: 50.8°C                     |               | β-Tocopherol (10 ppm)                       |       |
|                            | F: 1.5 kg/hr                  |               | γ-Tocopherol (352 ppm)                      |       |
|                            |                               |               | δ-Tocopherol (15 ppm)                       |       |
|                            |                               |               | Oleic (37.3%)                               |       |
|                            |                               |               | Linoleic (34.3%)                            |       |
|                            |                               |               | Palmitic (18.4%)                            |       |
|                            |                               |               | Stearic (3.9%)                              |       |
|                            |                               |               | α-Linolenic (0.5%)                          |       |
| SC-CO₂                     | P: 20-30 MPa                  | 6.22 – 16.17% | δ-Tocopherol (4.7 %)                        | [28]  |
|                            | T: 40 - 80°C                  |               |                                           |       |
|                            | F: 5 mL/min                   |               |                                           |       |
| SC-CO₂                     | P: 400 bar                    | 108.74%       | Phytosterol (7262.80 mg/kg)                | [29]  |
|                            | T: 40°C                       |               |                                           |       |
|                            | F: 20 mL/min                  |               |                                           |       |
| Soxhlet                    |                               | 146.0 g/kg    | Phytosterol (5072.4 mg/kg)                 | [29]  |
|                            |                               |               | β-sitosterol (78%)                          |       |
|                            |                               |               | Campesteryl (13%)                           |       |
|                            |                               |               | Stigmasterol (5%)                           |       |
|                            |                               |               | Cholesterol (2%)                            |       |
|                            |                               |               | Δ5-Avenasterol (2%)                         |       |
| Screw press                | Rotational speed:             | 70.58%        | Linoleic acid (37.53%)                     | [30]  |
|                            | 35 – 135 rpm                  |               | Oleic acid (30.47%)                        |       |
|                            | Tpre-heat: 45°C               |               | Palmitic acid (19.55%)                     |       |
|                            |                               |               | Stearic acid (6.78%)                       |       |
| Soxhlet (n-Hexane)         | T: 65 - 70°C                  | 19%           | α-Tocopherol (78 nmol/g)                    | [26]  |
|                            |                               |               | γ-Tocopherol (147 nmol/g)                   |       |
|                            |                               |               | δ-Tocopherol (4 nmol/g)                     |       |
|                            |                               |               | Linoleic (40.10 %)                          |       |
|                            |                               |               | Oleic (28.67%)                              |       |
|                            |                               |               | Palmitic (20.03%)                           |       |
|                            |                               |               | Stearic (5.28%)                             |       |
|                            |                               |               | α-Linolenic (0.66%)                         |       |
| Soxhlet (Petroleum ether)  | T: Boiling point              | 20%           | α-Tocopherol (27.54 mg/100 g)               | [31]  |
|                            | Time: 6 hours                 |               | β-Tocopherol (0.20 mg/100 g)                |       |
|                            |                               |               | γ-Tocopherol (67.58 mg/100 g)               |       |
|                            |                               |               | δ-Tocopherol (0.89 mg/100 g)                |       |
|                            |                               |               | α-Tocotrienol (2.11 mg/100 g)               |       |
|                            |                               |               | β-Tocotrienol (0.52 mg/100 g)               |       |
|                            |                               |               | γ-Tocotrienol (0.85 mg/100 g)               |       |
|                            |                               |               | Linoleic (37.11%)                           |       |
|                            |                               |               | Oleic (33.08%)                              |       |
3. Phytochemicals in roselle extracts
Many constituents have been reported previously present in roselle calyx and seed. This review will focus on three major bioactive constituents that often reported, anthocyanin, fatty acids and tocopherol.

3.1. Anthocyanin
Roselle calyces are known to be rich with anthocyanin. Anthocyanin can be present as red, purple, violet, orange and blue colour pigments [33]. It is the most important group of water-soluble pigments in plants. High level of the anthocyanin has been reported in fruits such as berries, grapes and some tropical fruits; and in leafy vegetables, grains and roots [34]. The most abundant anthocyanin is the glycoside form of delphinidin, cyanidin, petunidin, peonidin, malvidin, and pelargonidin [35]. Besides the application in the food industry, anthocyanin is proven to possess many health benefits, including prevention and treatment of a metabolic disorder. The antioxidant and anti-inflammatory properties have brought this bioactive compound to many advantages [36].

In Table 4, four important anthocyanins present in roselle calyces extract are compared with other pigment-rich materials that have been used in the food industry. Most of the major compounds in roselle calyces has not been found in other extracts, except for delphinidin 3-sambubioside and cyanidin 3-glucoside. Total anthocyanin in the calyces is high compared to others, means that almost all of the pigment comprised of anthocyanin, which in line with Tsai et al. [17].

Table 4. Comparison of roselle calyx extract with other commercialized seed oil in terms of anthocyanin content

|                    | Roselle | Blueberry | Corn Bran | Grape | Purple Sweet Potato | Red Cabbage |
|--------------------|---------|-----------|-----------|-------|---------------------|-------------|
| Total anthocyanin content (mg/g) | 58.60   | 5.19      | 3.625     | 32.80 | 102.31              | 3.00        |
| Delphinidin 3-sambubioside (mg/g) | 4.11    | -         | -         | -     | -                   | -           |
| Delphinidin 3-glucoside (mg/g)     | 0.15    | 3.64      | -         | 0.60  | -                   | --          |
3.2. Fatty acids
The seed oils are varied in fatty acid composition but most of them are rich in mono-unsaturated fatty acid (MUFA) or polyunsaturated fatty acid (PUFA) [44]. Most of the seed oil contain n-3, n-6 and n-9 fatty acids which generally called as omega-3 (ω-3), the alpha-linolenic acid, omega-6 (ω-6), the linoleic acid and omega-9 (ω-9), the oleic acid. Omega-3 fatty acid plays a significant role in maintaining the growth and functions of nervous tissue whereas omega-6 together with oleic acid, omega-9 monounsaturated fatty acid, balance the cholesterol level, increase immune ability and maintain heart health.

Table 5 shows the comparison of roselle seed oil with other commercialized oil in terms of fatty acids. The oil used for comparison are famous for their properties of high nutritional value, high oil quality and popular for health care use such as in cooking purpose, as health supplement and personal care product applications. Argan oil, grapeseed oil and flaxseed oil are mostly imported from overseas while extra virgin olive oil and virgin coconut oil are mostly produced and widely used locally. Roselle seed oil has almost the same fatty acid composition as argan oil which both are rich in oleic and linoleic acids with slightly more linolenic acid than argan oil. While compared with grapeseed, flaxseed and extra virgin olive oil, which are rich in omega-6, omega-3 and omega-9, respectively, roselle seed oil shows a balanced unsaturated and polyunsaturated fatty acid composition. Virgin coconut oil contains mostly saturated fatty acid. The findings on the content of linoleic and oleic acid of the roselle seed oil are useful to be applied as nutritional oil.

Table 5. Comparison of roselle seed oil with other commercialized seed oil in terms of fatty acids content (%)

| Oleic C18:1 | Roselle | Safflower | Rapeseed | Sunflower | Soybean | Grape |
|-------------|---------|-----------|----------|-----------|---------|-------|
| Linoleic C18:2 | 39.3    | 73.9      | 19.7     | 63.2      | 51.5    | 74.7  |
| α-Linolenic C18:3 | 7.3     | 0.1       | 9.6      | 0.1       | 7.3     | 0.2   |
| Saturated Fatty Acid | 21.5    | 9.7       | 9.6      | 12.0      | 15.6    | 10.4  |
| Unsaturated Fatty Acid | 78.5    | 85.5      | 87.1     | 83.6      | 79.7    | 89.7  |

Source: [44-46]

3.3. Tocopherol
The most attractive nutritional value of roselle seed oil is the tocopherols (vitamin E) content. Vitamin E is a group of fat-soluble compounds that cannot be synthesized in the body but to obtain only from foods and supplements. It was found to have distinct antioxidant activities and it is essential for the human body health [47]. The eight naturally occurring forms of Vitamin E are namely, the alpha (α), beta (β), gamma (γ) and delta (δ) classes of tocopherol and tocotrienols. These derivatives having the biological activity of alpha-tocopherol and have been found to possess vitamin E activity [48].

Table 6 shows the comparison of roselle oil with other commercialized vegetal oils. From the table, it is very obvious that roselle seed oil contains the highest alpha, gamma and delta tocopherols among all the commercialised oil. The tocopherol in roselle seed oil is four times the concentration in safflower and 20-fold higher than that in grapeseed oil, with up to 25% of alpha tocopherols, 5% delta tocopherols and 70% of gamma tocopherol in total tocopherol content [26]. Roselle seed oil presents the highest antioxidant properties. It is a very rich source of Vitamin E for edible oil, supplements as well as for natural preservative purpose. Besides, the high nourishing and strong antioxidants...
properties draw the attention of the personal care industry as it has high potential to work as a moisturizing agent and active ingredients in skincare products.

Table 6. Comparison of roselle seed oil with other commercialized seed oil in terms of tocopherols (mg/100 g of oil)

|       | Roselle | Sunflower | Rapeseed | Grape | Soybean | Jojoba | Hempseed | Argan |
|-------|---------|-----------|----------|-------|---------|--------|----------|-------|
| Alpha | 69      | 48.7      | 33.1     | 24.4  | 10.1    | 8.5    | 4.3      | 4.2   |
| Gamma | 153     | 5.1       | 39.3     | 2.8   | 59.3    | 33     | 61.5     | 32.3  |
| Delta | 24      | 0.8       | 0.8      | 1.0   | 26.4    | 0.02   | 3.32     | 1.83  |

4. Future perspective as a health supplement

The potential of roselle calyces and seeds in pharmaceutical industries has been previously discussed [16,49-51]. Roselle colour has been used in tablet coating, toothpaste, ointments, etc. to increase the appearance of the products. The minerals content and pharmacological properties of anthocyanin provide the benefits when applying as a health supplement and in the human diet as they possess antioxidant, anticancer, antibacterial, treat high blood pressure, and more. Meanwhile, Vitamin C content in the calyces’ extract can be the added value in the supplements. Meanwhile, roselle seed oil can be an important source of natural dietary antioxidants, since it is rich in omega-3 and 6 which cannot be synthesized by the body. The high fibre content of the oil also can be fully utilized as a meal replacement.

The high-value local source, roselle plant, is not fully utilized. The usage of roselle in various industries, as well as in health supplement, will bring a great impact both in economic and social in Malaysia. Besides, as the roselle-based production increase, it is able to elevate the agricultural industry by increasing the roselle plantations. There will be more working opportunities open to populations.

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