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ORIGINAL ARTICLE

Study of the Nutritional, Antimicrobial and Chemical Properties of *Hibiscus sabdariffa*: Towards Findings Novel Natural Substance for Active Film

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ABSTRACT: Recent studies have shown that *Hibiscus sabdariffa* has many pharmacological properties, including antioxidant and antibacterial activity. Roselle, which is widely used in different industries, is a promising pigment because of a lot of anthocyanins, which can show color changes in response to different pHs. This study aimed to investigate the chemical compounds of roselle flower, including ascorbic acid, mineral content, total sugar, and fatty acids and chemical properties such as moisture content, acidity and total soluble solids. Ascorbic acid and total sugar content were performed by HPLC. *Hibiscus sabdariffa* was observed to be rich in monosaccharides, especially glucose (9.22 g kg^{-1}) and fructose (3.18 g kg^{-1}), but low in maltose. The results of the chemical properties of roselle showed that the moisture content, total soluble solids, and acidity of roselle flower are 13.23%, 6.35%, and 4.98%(g malic acid(100 mL)$^{-1}$), respectively. The analysis of mineral content showed that among the nine elements, calcium had the highest concentration. The fatty acids of roselle include stearic, palmitic, oleic, linoleic, and linolenic acids. The antimicrobial properties of the ethanol and water extracts of roselle showed that as the concentration of the extract increased, the antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, and Escherichia coli* significantly increased ($p<0.05$). The obtained results could be beneficial for introducing interesting characteristics of the roselle such as rich resources from anthocyanin pigment and antimicrobial properties and can be used for active packaging.

INTRODUCTION

Flowers, leaves, and fruits are widely used in many countries, and they have positive effects on consumers' health [1, 2]. Medicinal plants have constant activity against diseases such as allergies, chronic pains, high blood lipids, cancer, arthritis, and heart diseases and have minimal negative effects [3, 4]. The *Hibiscus sabdariffa* L., also known as Jamaica flower, Abyssinia, and hibiscus, belongs to the Malvaceae

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family. The species is native to Africa and produces flowers annually. *Hibiscus sabdariffa* flowers are composed of red-purplish calyx and corolla. Roselle is grown in tropical regions around the world such as India, Philippines, Malaysia, Senegal, Ethiopia, and Mexico [5]. Due to its distinctive freshness, roselle provides a beverage that is typically known as red tea and is well-known around the world. Roselle products are commonly used in traditional medicine to obtain antihypertensive effects and to prevent heart and vascular diseases [6]. The petals of the red flower are potentially a good source of antioxidant agents such as anthocyanins and ascorbic acid. Food products such as drinks and vegetables or dietary supplements such as vitamins are often used preventively due to their immediate effect on the body's fluid rejuvenation [7].

Anthocyanin colorant of roselle has been used as a nonartificial pigment in many foods (Rodriguez-Amaya, 2016).

The use of *Hibiscus sabdariffa* is common in food products, because of its antioxidant activity in humans, especially against chronic diseases, it is used to prepare hot and cold drinks [8]. The mentioned effects are related to the presence of some phenolic compounds such as anthocyanins (cyanidin derivatives), flavonols (quercetin and kaempferol derivatives), phenolic acids (chlorogenic acid), and also due to the presence of a specific organic acid of *H. sabdariffa* [9]. Anthocyanins are natural colorants and they can indicate color changes in response to pH variations over a wide range. There have been more studies recently that aim to estimate pH indicators utilizing anthocyanin pigments. *Hibiscus sabdariffa* is a herb whose calyx is highly anthocyanin-rich [10]. Roselle is a good resource ascorbic, tartaric, oxalic, malic, citric, stearic, arachidic, protocatechuic, and delphinidin acids, cyanidin, betacarotene, betasitosterol, galactorrhea, pectin, quercetin, thiamine, riboflavin, niacin, and minerals such as potassium, phosphorus, and iron [11]. Therefore, the extract of red flowers can also act as an antioxidant for example, it protects against low-density lipoprotein (LDL) oxidation and has lipid-lowering effects inside the body. The most popular way to use roselle is to make scented red-purple tea by extracting a soluble solids substance with hot water. Extracts are also used as a flavor for sauces, jellies, marmalades, and soft drinks and as colorants for foods [12]. The methanolic extract of roselle flower has been investigated for phytochemical compounds, antimicrobial activity, and cellular toxicity that proximate compositions of *H. sabdariffa* include glycosides, flavonoids, saponins, and alkaloids. Antibacterial activity was demonstrated against *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Serratia marcescens, Clostridium sporogenes, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, and Pseudomonas fluorescens* under laboratory conditions [13]. However, less information is available on the antimicrobial effects and chemical properties of roselle in various extracts. Therefore, the study aims to determine the nutrient compounds of roselle, including ascorbic acid, mineral compounds, sugar, and fatty acids, and antimicrobial properties against *Bacillus subtilis, Staphylococcus aureus, and Escherichia coli* as a herbal tea.

MATERIALS AND METHODS

Roselle flower

The dried *Hibiscus sabdariffa* L. harvested in November 2022 in Iran was obtained from the producer from Khorramshahr.

Chemical properties

Sugar and ascorbic acid were analyzed using high-performance liquid chromatography (HPLC), according to the Previous works with a slight modification[14, 15]. The moisture content of roselle was evaluated according to AOAC (2000). To evaluate the pH, 5 g of roselle calyx powder was added to 5 Ml of distilled water, shaked for 30 min, and filtered through filter tissue (Whatman No. 1). The pH was evaluated using a pH meter (744 metrom Genway ENG). The total soluble solids were evaluated using a refractometer (RFM 330 Carl Zeiss Jena, GER). Acidity was measured using the titration method until reaching a pH 2.8. To determine minerals, 1 g of the specimen was placed in a microwave tube and 8 ml of 70% nitric acid was added to the tube, for 25°C at 1 hour. The sample was heated on a hot plate and cooled.

Elements were determined by inductively coupled plasma (ACTIVA, Jobin Yvon, FRA). To assess fatty acids, crude fat was first extracted using the Soxhlet system. Fatty acid methyl esterification was completed using a methanol-BF3 reagent before gas chromatographic analysis (Kenauer, Livogen pharmed, GER).

Roselle flower extract

Roselle extract was prepared according to Giusti, et al. [16] with a slight modification. About 1 g of roselle calyx powder was added to 16 mL of 80% ethanol and the pH of mixture was controlled to 2 with HCl. The specimens were heated for 60 min at 50°C and centrifuged at 3000 rpm for 5 min to get the final extract. The solvent was separated with a rotary evaporator (Heidolph Hei-VAP, GER) at 50°C. About 20 g of dried roselle calyx powder was added to distilled water (1:40w/v) and extracted for 16 min and at 100°C (water extract). Extraction ratio were measured according to moisture content of dried roselle sample. After extraction, the specimen was filtered using filter tissue (Whatman No. 4.)

Antimicrobial characterizations

The antimicrobial effect of the specimens on different microorganisms was evaluated according to pervious scholars with slight modifications [17]. *Bacillus subtilis* gr +(ATCC 6633) was cultured at 30–35°C for 16 h ; *Escherichia coli* gr-(ATCC 8739) and *Staphylococcus aureus* gr+(ATCC 6538) were cultured at 37–35°C for 16 h in Tryptic Soy Broth (10mL), respectively. To estimate the growth inhibition, each microorganisms was grown in favorable media and diluted (OD at 660 nm = 0.04) by UV-visible spectrophotometer (UV 1650PC, Shimadzu, Tokyo, Japan). Control and diluted specimens were absorbed on sterilized paper discs and were placed on the plate, which was incubated for 16 h.

Statistical analysis

One-way ANOVA and Duncan's tests were used for antimicrobial properties and different parameters based on different extracts at a significant level of 5%. Analysis was done using graph pad Prism 6 soft ware.

RESULTS AND DISCUSSION

Ascorbic acid, Sugar content, and chemical properties of roselle

Table 1 shows the proximate compositions, including various types of sugars and chemical properties of roselle. The findings showed that the most common sugar content of *Hibiscus sabdariffa* is glucose, followed by fructose as the second most common sugar content. Another study also showed that roselle has the highest amount of glucose, which is consistent with the findings of the presentation study [12].

Roselle Ethanol extract showed that carbohydrates are the main macro-molecular and glucose and fructose are the main sugar contents of the extract, with concentrations of 5.6 and 6.4 g/dry weight, respectively. According to other researchers, these monosaccharides demonstrate long-term performance benefits in sports due to polysaccharides induce a spectrum of effects on muscle metabolism and are likely to improve performance [18, 19].

Ascorbic acid has various applications in the food industry, such as preventing enzymatic browning and color loss, antioxidant ingredient of oils and fish, and dairy products, and color stabilization of meat products [20]. The daily recommended intake limit of ascorbic acid according to RDA in the United States is 60 mg day^{-1} for adults, with an average intake of 46 mg day^{-1} to prevent scurvy [21]. Roselle is a rich source of ascorbic acid, in this study, vitamin C content of roselle was 3.58 mg k^{-1}. In another study, ascorbic acid content of *Hibiscus sabdariffa* was 2.47 mg k^{-1} [12]. It should be noted that ascorbic acid is highly sensitive to light, oxidizing agents, pH, high and, oxygen. When metals like copper are present, high temperatures can degrade vitamin C, and the conditions and region of the plant's production can have an impact on its close-proximate compositions [22].

The results of the chemical properties of *Hibiscus sabdariffa* showed that the soluble solids, acidity, and moisture content of roselle are 6.35%, 4.98 g malic

acid/100ml 13.23% respectively. The results obtained were consistent with other studies that reported acidity level of 4.2 g malic acid/100ml, pH = 1.2, moisture content = 11.08%, and a soluble solids content = 45.5%. Also, the moisture content of the roselle was 1.87%. These factors were also investigated in alcohol and water extracts, which showed that both types of extracts have high acidity, low pH, and low soluble solids content. The acidity level was the same in both extracts, but the pH of the the water extract was lower than that of the alcohol extract, and the soluble solids content of water extract was higher than that in the alcohol extract [12]. Comparatively similar findings were found in the work of conventional, ultrasound- assisted, and microwave extraction of active ingredients from *Hibiscus rosa-sinensis* [23]. This study indicated that 2% citric acid solvent in ultrasound-assisted extraction, 85% ethanol solvent +conventional extraction, and distilled water solvent in microwave-assisted extraction had higher titrable acidity, total soluble solids, and pH than the other methods

Table 1. Ascorbic acid, suger content and chemical properties of roselle.

Compositions	Content
Glucose(g kg^{-1})	22.9±0.18
Fructose(g kg^{-1})	18.3±0.12
Maltose(g kg^{-1})	0.03±0.001
Sucrose(g kg^{-1})	4.3±0.15
Ascorbic acid(mg kg^{-1})	58.3±0.28
Acidity(g malic acid(100$ml)^{-1}$)	4.98±0.2
pH	3.2±0.16
Total soluble solids (%)	6.35±0.33
Moisture content (%)	13.23±0.2

Data are mean and SD.

Elements and fatty acids of roselle

Appropriate levels of vitamin D_3 and calcium are important factors in maintaining the functional properties of organs and physiological performance of the body [24]. Calcium intake is associated with reducing obesity and its related risks and complications in all epidemiological studies. Additionally, it has been shown that calcium supplementation is effective to reduce weight in obese individuals. Therefore, acute calcium intake has been reported to be associated with fat oxidation of the human body [25].

It is usual for people of all ages, including children and the elderly, to consume these substances insufficiently, and the recommended calcium intake requirement can change based on age, gender, and ethnicity. Moreover, vitamin D_3 affects calcium absorption and the optimal amount is needed for calcium to be absorbed and used effectively[26].

Therefore, roselle can be introduced as a rich source of calcium for bone-related diseases and chemical analysis of the mineral content of *Hibiscus sabdariffa* has shown high calcium content (Table 2). In another study, it was stated that the calcium content of the flower is 10707.58 mg k^{-1}, which was introduced as the highest mineral content of the flower, which is consistent with the present study [12]. Also, the mineral content of three types of *Hibiscus sabdariffa* calyx, including green, red, and dark, was evaluated. It was reported that the content of calcium, magnesium, potassium, and sodium in dark red color was very high, and dark red flowers showed a significantly higher content of iron compared to other samples. This type of flower can be useful in blood disease. The highest mineral in all three types of flowers was potassium, followed by calcium [27].

The calcium content of roselle found in this study was higher than the calcium content of roselle grown in Cameroon (512 mg(100g) $^{-1}$) and Egypt (912 mg $(100g)^{-1}$). The calcium content observed for roselle was higher than the calcium content of Omija (7138.38 $mg(100\ g)^{-1}$) [28].

In several studies, linoleic acid has been mentioned for its various biological activities, including reducing body fat, preventing arterial hardening, increasing bone health, reducing cholesterol and blood pressure levels, and improving immune function [29, 30]. According to Table 2, roselle has a high concentration of linoleic acid, followed by a high concentration of palmitic acid. Jung et al., 2013 showed that the highest amount of fatty acid in roselle belongs to linoleic acid, and the lowest amount of fatty acid belongs to linolenic acid [12]. Similar findings were observed in the study of roselle calyces from Guinea-Bissau. This study stated that hydroethanolic extracts of roselle containing major fatty acids such as palmitic acid, followed by linoleic and oleic acids [31]. *Hibiscus cannabinus* seeds have significant amounts of oil (18.14%), and their fatty acids include palmitic acid (20.75%), oleic acid (28.91%), vernolic acids (4.16%), stearic acid (3.96%), linoleic acid (39.49%), and dihydrosterolic acids (1.08%). However, *Hibiscus sabdariffa* seeds have the appropriate level of oil (17.35%), palmitic acid (18.52%), oleic acid (25.16%), vernolic acid (3.52%), stearic acid (4.31%), linoleic acid (44.72%), and dihydrosterolic acids (1.57%). For edible oils, a high ratio of oleic/linoleic acid and lower level of dihydrosterolic acids are preferred, for industrial oils, high level of vernolic acids is preferred [32].

Table 2. Elements and fatty acids content of roselle

Mineral	Content (mg kg^{-1})
As	0.003±0.00
Cr	0.45±0.02
Cu	3.68±0.12
Ca	10809.3±9.15
Na	455.3±7.28
Mn	259.09±1.9
Al	123.35±0.35
Fe	168.12±3.63
Zn	33.13±0.95
Palmitic acid	7.18±0.14
Stearic acid	2.53±0.18
α-linolenic acid	2.85±0.21
Linoleic acid	7.52±0.43
Oleic acid	2.98±0.18

Data are mean and SD.

Antimicrobial properties

Antibacterial, anticancer, and antioxidant properties of essential oils, extracts and nanoparticles led to their usage as novel pharmaceutical substances and as an antimicrobial food additives [33-35].

Table 3 shows the antimicrobial properties of the hydro-alcoholic extract of *Hibiscus sabdariffa* against *Bacillus subtilis, Staphylococcus aureus, and Escherichia coli.*

Increasing the extract concentration enhances antimicrobial properties significantly against microorganisms ($p<0.05$). The highest antimicrobial activity was observed at 200 mg mL^{-1} against *Staphylococcus aureus*, while *Bacillus subtilis* showed the highest resistance to the extract.

Table 3. Antimicrobial properties of roselle hydroalcoholic extract.

Concentration (mg mL^{-1})	*B. subtilis*	*S. aureus*	*E.coli*
75	13.8.±0.16c	27.3.±0.08c	15.84.±0.23c
150	16.85±0.19b	32.3 ±0.34b	20.6±0.35b
200	19.81±0.22a	35.22±0.28a	24.8±0.17a

Data are mean and SD. Different letters in column show significant difference at $p<0.05$ among roselle hydroalcoholic extract

Table 4 demonstrates that the use of the highest concentration of extract has a significant impact on microorganisms ($p<0.05$). The final findings showed that the highest antimicrobial activity of aqueous extract belongs to *Staphylococcus aureus*.

Table 4. Antimicrobial properties of roselle water extract

Concentration (mg mL^{-1})	*B. subtilis*	*S. aureus*	*E.coli*
75	12.5.±0.2c	25.17.±0.14c	14.8.±0.15c
150	15.65±0.15b	30.09±0.22b	17.38±0.24b
200	19. 2±0.13a	33.5±0.35a	21.09±0.2a

Data are mean and SD. Different letters in column show significant difference at $p<0.05$ among roselle water extract.

In recently study, hydro-alcoholic extract of roselle demonstrated that it has the same antibacterial effect on different microorganisms, but the lowest minimum inhibitory concentrations (MIC) were reported against *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Enterobacter cloacae, Pseudomonas aeruginosa, and Salmonella typhimurium* [36]. In another study on the antimicrobial properties of *Hibiscus sabdariffa*, it was shown that the highest MIC were against *Micrococcus flavous, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa*, and *Salmonella typhimurium*.

The findings were similar to previous works that investigated the antimicrobial properties of Roselle calyces against foodborne microorganisms. Previously, it was stated that hydroethanolic extract of roselle inhibited the growth of *Micrococcus flavus, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa,* and *Salmonella typhimirium* [31].

The obtained finding indicates that the extract has a high antibacterial potential [2]. The cytoplasmic membrane of microorganisms is damaged by the alcohol extract of roselle, which also disturbs the membrane's lipid structure and alters its permeability properties and membrane functions [37-39]. Katalinic, et al. [40] reported that membrane permeabilization leads to increased proton leakage from the cell, disruption of the membrane potential, and proton motive force, subsequently reducing adenosine triphosphate (ATP) synthesis. Can Baser [41] suggested that the plasma membrane damage caused by the alcohol extract of roselle is associated with their hydrophobic properties. The various effect of the extract on microorganisms may be related to different polysaccharide layers of cell walls. Microorganisms with thicker outer layers of polysaccharides may not be penetrated by roselle extract at low concentrations, but the thick polysaccharide layer can be disrupted at higher extract concentrations [42, 43]. The high sensitivity of gram-positive bacteria against extract may be due to their single-layer cell wall, while in gram-negative bacteria, cell wall is composed of several layers. The antibacterial effect of flavonoids is thought to occur by the formation of complexes with the outer membrane and soluble proteins that are attached to the membrane [44, 45]. These compounds also induce antibacterial effects by penetrating the cell membrane. Phenolic compounds also interfere with the cytoplasmic membrane and the entry and departure of substances from cells to exert their antibacterial effects. Overall, different studies prove the antimicrobial effects of various species of roselle depending on the solvent type and application method. The findings suggest that roselle extract has favorable antibacterial properties and can be used in the food and pharmaceutical industries [2].

CONCLUSIONS

The presence of a variety of nutritional and bioactive compounds in medicinal plants enables them to provide different preventive and therapeutic actions, and create active packaging in food industry. The obtained results in the presentation work show that roselle has compounds such as linoleic acid, palmitic acid, calcium, sodium, glucose, fructose, and ascorbic acid. Also, the presence of phenolic acids causes chemical characteristics such as low pH and high acidity in roselle. In addition, the roselle extracts showed antibacterial properties against Bacillus subtilis, Staphylococcus aureus, and Escherichia

coli. The obtained findings show the potential *Hibiscus sabdariffa* to be used as an antimicrobial compound in the packaging and pharmaceutical industries

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Conflict of interests

The authors declare that there is no conflict of interest.

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