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Master's
Dissertation

Antioxidant activity of Ajwa date extract for cosmetic ingredient

Graduate School of Industrial Technology and
Entrepreneurship Chosun University

Department of Beauty and Cosmetology

Khlood Lafi Alharby

Antioxidant activity of Ajwa date extract for cosmetic ingredient

화장품 원료로 사용 가능 한 대추 야자 추출물의
항산화 활성

25th February 2022

Graduate School of Industrial Technology and
Entrepreneurship Chosun University

Department of Beauty and Cosmetology

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Advisor: Prof. Hyun-Jae Shin

This dissertation is submitted to the Department of
Beauty and Cosmetology and the Graduate School of
Industrial Technology and Entrepreneurship Chosun
University in fulfilment of the requirements for the
master's degree


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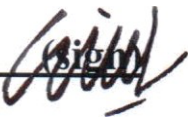
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**Graduate School of Industrial Technology
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ABSTRACT

Antioxidant activity of Ajwa date extract for cosmetic ingredient

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Ajwa대추 열매(DF)와 종자 Ajwa대추 열매(DFS)는 많은 건강상의 이점이 있는 페놀 화합물의 중요한 공급원입니다. 자유 라디칼 중화에 있어 DF와 DFS의 잠재력을 조사하기 위해 100% 에탄올, 70% 에탄올, 뜨거운 물 추출물의 세 가지 방법으로 추출한 결과 항산화제와 페놀 화합물의 양.

실험 연구에서 플라보노이드를 함유한 3가지 추출물 모두 항산화 활성이 있는 것으로 관찰되었으며, 이 활성은 농도 측면에서 각 추출물과 다른 추출물 사이에 차이가 있음을 알았습니다. 하이드록실 라디칼 패밀리에서의 활성 DPPH 뿌리 및 ABTS에 의한 항산화 테스트를 사용할 때.

또한 이들 추출물의 페놀화합물 함량이 서로 다른 것으로 나타났으며, 열수추출물(DF1)의 페놀화합물 함량이 최대 3008.66 μ g/g으로 가장 높았다.

본 연구를 통해 Ajwa대추야자의 열매와 종자는 약간의 생물학적 활성을 보인 천연화합물 측면에서 수익률이 좋다고 할 수 있으며, 효능과 활성면에서 차이가 나는 경우에는 좋은 것으로 볼 수 있다. 화장품의 성분.

Ajwa date fruit (DF) and Ajwa date fruit seed (DFS) are essential sources of phenolic compounds with many health benefits. To investigate the potentials of DF and DFS in neutralizing free radicals, they were extracted by three different methods: 100% ethanol, 70% ethanol, and hot water extract, and the results showed a difference between them in terms of the effectiveness of antioxidants and the number of phenolic compounds.

In the experimental study, it was observed that all three extracts containing flavonoids had antioxidant activity, and found that this activity differed between each extract in terms of concentration, where the 100% ethanolic extract of the seeds (DFS3) showed the most significant activity in the hydroxyl radical family, when using antioxidant assay DPPH and ABTS.

It was also found that these extracts had different amounts of phenolic compounds, and the hot water extract of the fruit (DF1) recorded the highest content of phenolic compounds, up to 3008.66 $\mu\text{g/g}$.

This study shows that the fruits and seeds of the Ajwa date palm have a good return in terms of natural compounds that showed some biological activities, and although they differ in terms of effectiveness and activity, they can be considered a good component of cosmetic products.

I . Introduction

1.1. Background of the study

1.1.1. Date palm tree

The date palm has accompanied the inhabitants of dry areas since ancient times and for different historical periods. The date palm grows in areas in the Middle East, North Africa, and the United States, and it is the only tree or plant mentioned in all the holy books (the Torah, the Bible, and the Qur'an). It has a long and rich record in history, through civilization and humanity, as most Arab writers and philosophers mentioned in their books, as well as in works of research and poetry, drawn from many ancient peoples in Mesopotamia, the Nile Valley, and the Indus Valley [7].

In the Arabian Peninsula, the date palm and camel are considered symbols in every time and place.

The date palm "*Phoenix dactylifera*" is one of the most widely consumed fruits in the world. Globally, 2000 varieties have been cultivated, and 10% have been described according to the fruit characteristics [8]. Saudi Arabia is a significant producer of date palms. Exports registered a 27% rise in the middle of 2019 Compared with 2018 [9], and the demand and production rate have increased rapidly. Date fruits are affordable and rich in fiber and energy, and are considered a staple food, along with grains, potatoes, and rice.

The developmental stages of dates have internationally adopted Arabic names according to their stages of maturity, such as Hababauk, Kimry, Khalal, Rutab, and Tamar (Fig. 1). The stage of maturity, yield, physical condition, flavor, texture, and nutritive value are affected by agricultural practices and climatic conditions [10].

The non-edible seed portion is usually discarded after consuming the date fruits. They may be scattered and sown in fields. Seed propagation is the easiest and quickest method of propagation and may yield hybrid seedlings. Date fruits consist of the epicarp, a fleshy mesocarp (pulp), and an endocarp consisting of a seed called a kernel or pit, which constitutes 10–15% of the date fruit weight [11].

The palm agroindustry and processing industries have generated vast amounts of seed waste and date fruit.

Additionally, a high production rate, improper handling, and lack of scientific knowledge may increase the wastage by more than 30% of the production values [12]. The agricultural wastes consist of cellulose, hemicelluloses, lignin, and others. Dates are a good source of sugar (70–80% sugar content), which varies according to the species and the fruits' maturity stage. The significant sugars in most date cultivars are glucose, fructose and sucrose, which are easily absorbed by the body to provide energy [13].

Dates are an indispensable source also of dietary metals and free amino acids and have been used to treat chronic illnesses and diseases since ancient times [14]. They have also been found to reduce high blood pressure and oxidative stress, and have been used as a treatment to reduce these conditions, as well as diabetes, cancer, and atherosclerosis, and to stimulate immunity [15]. Moreover, they contain several bioactive compounds, such as coumaric acids, ferulic acid, cinnamic acid, flavonoids, phenolic compounds, and vitamins [16].

Date fruits and seeds have been reported to possess anti-aging properties and overcome wrinkling of the skin in women. Date waste is believed to contain many essential components that strengthen the hair and skin, prevent early graying of hair, stop wrinkle development, and give the skin a fresh look [17].

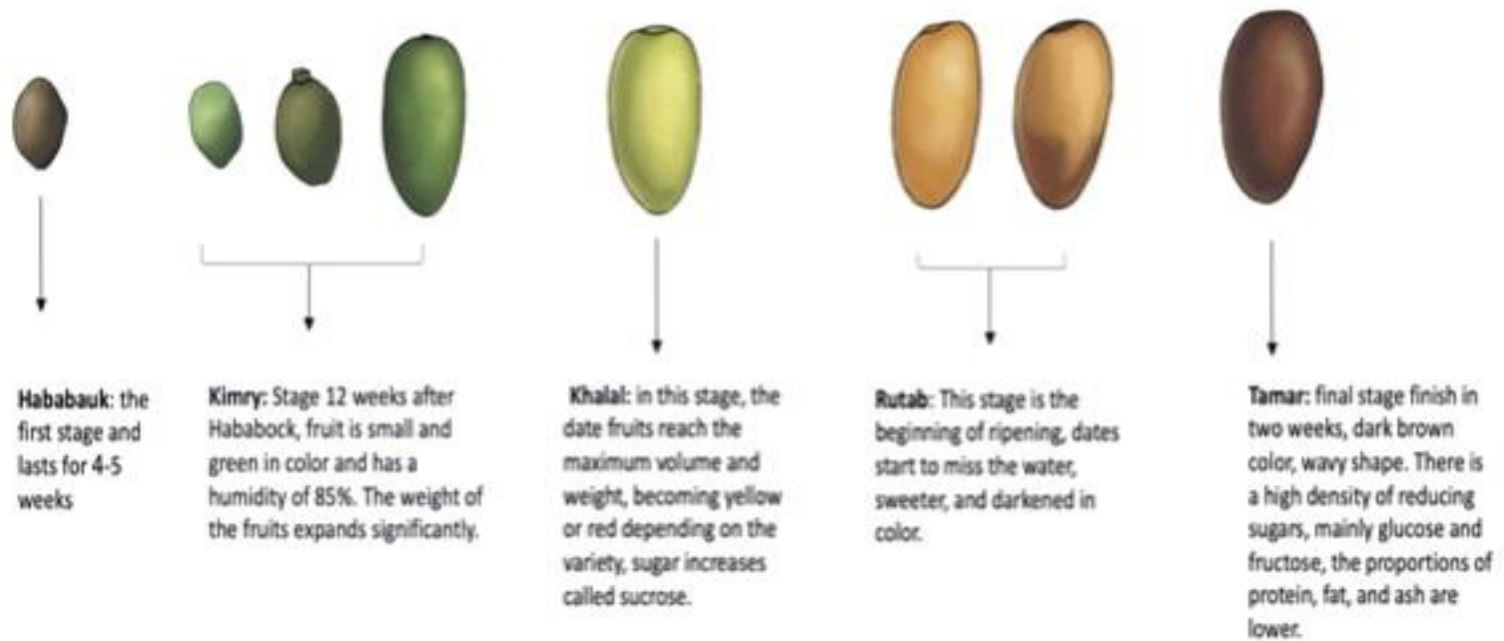


Figure 1. Date fruit stages of maturity.

1.1.2. Scientific classification

The scientific name of the date palm is *Phoenix dactylifera*. The palm tree is dicotyledonous, meaning that there is a palm bearing male flowers and another bearing female flowers that produce fruits.

In turn, it belongs to the palm family (*Palmaceae*) and to the genus known as (*Phoenix dactylifera* L.), which is derived from the word “Phoenix” meaning date palm in the *Phoenician*, and “*dactylifera*” from the Greek word “*dactulos*”, meaning finger.

Date trees usually reach a height of about 23 m, and they grow singly or form a clump with several stems from a single root system. The leaves are 3 to 6 meters long, with thorns on the petiole, and pinnate, with about 150 leaves. The leaflets are 30 cm long and 2 cm wide. The full extent of the crown of the date palm ranges from 4–10 m (Fig. 2).

The date palm is dioecious, having separate male and female plants. They can quickly be grown from seed, but half of the palm will be female, and therefore date production will be affected because half do not bear fruit, and the dates are often smaller and of poor quality. Thus, most farmers make intensive use of cuttings of crop varieties. Plants grown from cuttings will produce females as soon as 2–3 years from seedlings.

Dates are naturally pollinated by the wind but are pollinated entirely by hand on modern commercial farms. Natural pollination occurs with an equal number of male and female plants. However, with the help of manual pollination, one male can inseminate up to 100 females. Since males are only valuable as pollinators, farmers can focus their resources on the female palms producing the fruit.

Moreover, some growers do not even keep any male plants, as male flowers are available in local markets during the pollination season. Pollination is done manually by workers or with a wind machine [18].

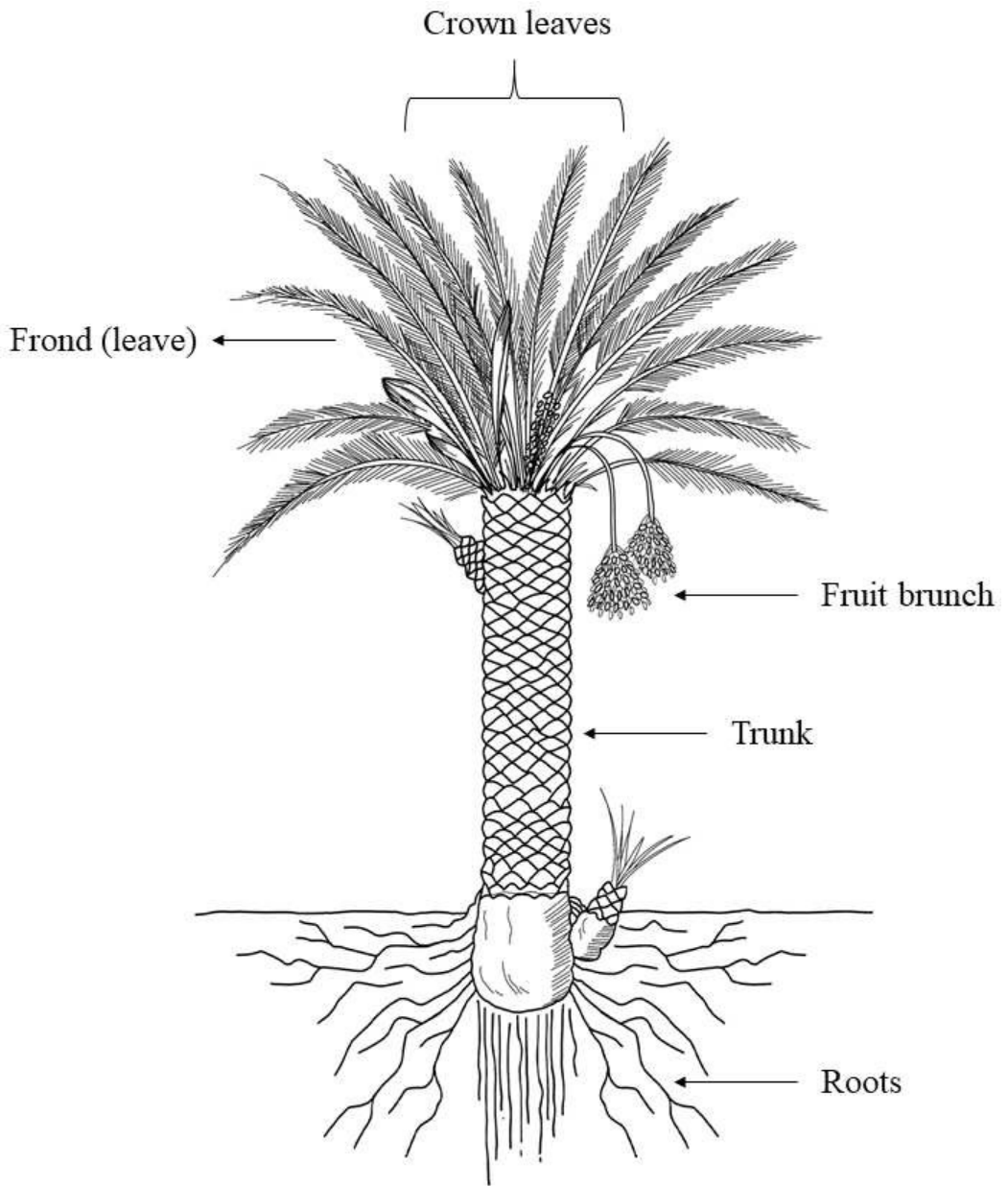


Figure 2. The main parts of the date palm tree.

1.1.3. Economic importance

The desert region is characterized by two basic phenomena, namely desertification and drought, but the date palm can tolerate this, so there is a comprehensive awareness of the importance of the nutritional value of dates as an integrated food source that achieves self-sufficiency due to its nutritional content of sugars, vitamins, and mineral salts. In addition, everything in the palm tree has a use. It is one of the main pillars of economic and social stability in the agricultural areas due to its longevity and strength, its origin, and the importance of its products (Fig. 3).

Palm trees have played an essential role in the Gulf region's environment due to their wide use also in landscaping [19].

The tree consists of different parts, such as the leaflet, rachis, and fibers, and each palm tree produces an average of 15 new leaves every year. The surface fibers and additional waste fruit clusters (10–15 kg) create a total of 40 kg of waste per tree [20], and many different kinds of traditional, natural, and manufactured materials have been developed from date palm waste, such as mats, screens, baskets, crates, fans, walking sticks, brooms, fishing floats, and fuel.

Moreover, the leaf fiber and sheaths are used to prepare packsaddles, rope, coarse cloth, and large hats [21]. The date industries have been looking towards developing value-added products, as they are highly profitable and may reduce the risk of date deterioration.

Many studies indicated a high potential for converting date palm waste into energy while eliminating the polluting impact on the environment and the cost associated with the disposal of this waste [22]. In addition, date fruit extract, paste, and fermented products are used for making dietary supplements, juice concentrate, syrup, yogurt, bread, confectionery, and preservatives [23-24]. Date fruits are also used in the production of high-quality fruit wine [25]. Date seeds constitute a surplus of date fruit by-products due to processing, and their exploitation in food and feed provides an economic advantage [26]. In addition, more than 127,000 metric tons of seed oil is extracted annually from date kernels, from which fatty acids and oils are derived and used in the cosmetic industries [27,28]

Seed essential oil is suitable for use in the production of soaps/moisturizing creams, shampoos and other skincare products [29,30]. Moreover, it is rich in fibers and phenolic compounds and exhibits high oxidative stability [31-32]. These include dyes, dietary fibers, organic acids, sugars, and antibacterial or antifungal compounds, which have shown health-promoting effects in humans [33,34].



Figure 3. Traditional products using different parts of the palm tree.

1.2. Antioxidants

Antioxidants provide a defensive system to protect the cells of the body from damage. They consist of some enzymes made by the body and some nutrients that a person eats within his daily food. After their formation, resistance, and conversion to another form, free radicals lose the ability to oxidize. A free radical is a molecule or atom that contains in the outer orbit a single electron (OHo, O, ClOo, ONOOo, NOo, ROo, ROOo, HOOo, No, HO), and this makes it try to restore the lost 22 electrons from other body compounds, which causes damage to body cells by breaking the protective barrier that surrounds the cells during its interaction with the phospholipids of the cell membranes, which leads to damage to everything from DNA to the collagen layer in the skin.

1.2.1. Definition

An antioxidant is any substance present in the lowest concentration relative to that of an oxidizable substrate, which delays or prevents the oxidation of this substrate [1]. The term “oxidizable substrate” includes all substances found in living cells, such as proteins, lipids, hydrocarbon compounds, and nucleic acids. Antioxidants can act by several different mechanisms: direct deletion of oxygen, the capture of reactive oxygen or nitrogenous species NOS and ROS, inhibition of the formation of ROS and NOS species, removal of the metal ions necessary for ROS formation, or induction of internal antioxidant defense [1]. The terms “active oxygen” and “nitrogen species” include all the oxygen- or nitrogen-bearing radicals and some non-radical compounds that are oxidizing agents or are easily converted to radical elements. This second type contains especially hydrogen peroxide and peroxyxynitrite.

1.2.2. Family of molecules with antioxidant activity

The cellular defense systems against radical attacks consist of special enzymes (SOD, GPX, CAT) and “small” molecules of a food source such as vitamin C or vitamin E,

and other compounds. Date is a food source with significant antioxidant activity, including carotenoids and phenolic compounds that contain phenolic acids, flavonoids, and tannins.

1.2.3. Environmental causes causing the formation of free radicals

These include:

- Ionization rays from industry.
 - Exposure to sunlight and cosmic rays.
 - Ozone, car exhaust, heavy metals (mercury, cadmium, lead, other chemicals).
 - Smoking.
 - Alcohol abuse. Trans fats, chemicals that pollute water, air, food, and pesticides
- (Fig. 4).

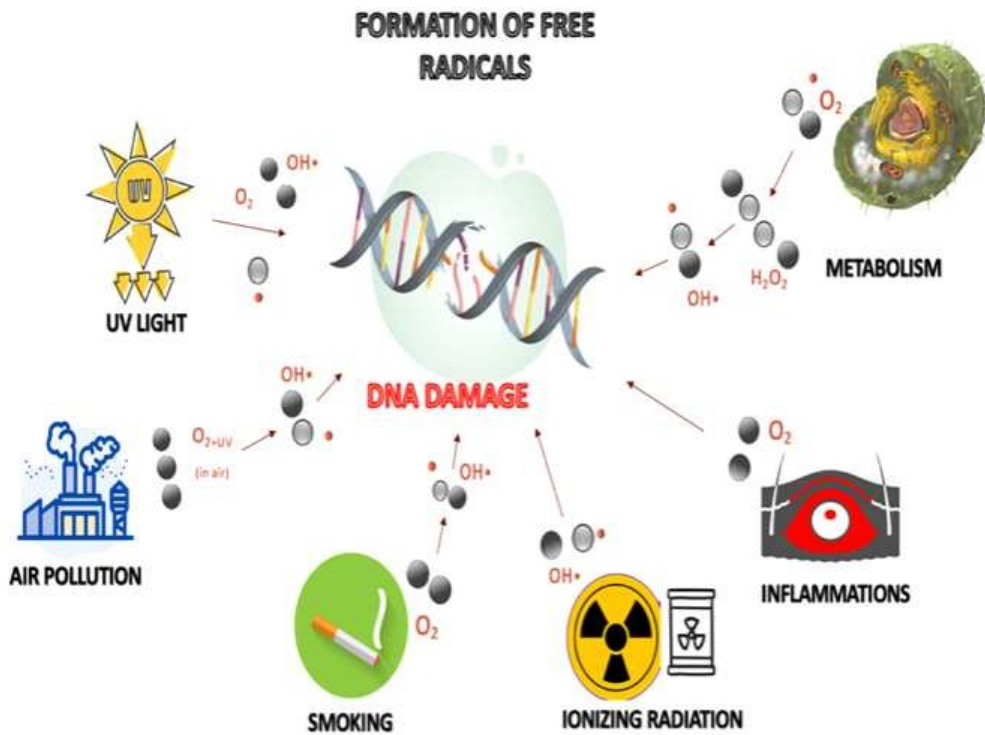


Figure 4. Environmental factors causing the formation of free radicals.

1.3. Research trends and composition

1.3.1. Research trends

The study and identification of botany are essential because human life has become closely related to the life of plants as food. In addition to using plants as food, humans have associated many of the plants that cover the Earth's surface with diseases that afflict them and used these plants or parts of them in medicine.

Nowadays, many developed countries have focused on the importance of plant extracts, and the pharmaceutical and biological industries are thriving due to the discovery of the benefits of plant extracts [1].

The importance of plant compounds that have medicinal properties derived from the lives of peoples in the past and, with the discovery of pure and effective compounds from plants in the nineteenth century as a result of the development of chemical sciences and laboratories, the use of natural extracts continued and became a large part of the molecular sciences that have led to the identification and extraction of many compounds such as polyphenols.

In America, 25% of medicines are herbal, so a green product free of synthetic chemicals has become generally acceptable to people [2].

Moreover, most of a plant contains one or more chemicals in small or great concentrations either in their natural form or through the active chemicals extracted from it, and can treat one or more specific diseases or reduce the symptoms of the disease.

In addition to the pharmaceutical industry, there is a new industry emerging in the world of beauty, known as “green cosmetics”. The market for natural and organic skin and body care products is worth over \$50 billion and has grown at an average of 6% annually since 2007 [3].

People have been interested in beauty and external appearance since ancient times, and because the effects of many annoying external factors have led to the appearance of melasma, wrinkles, hair loss, or growth in undesirable places, and infection with skin diseases. Humans are keen to find treatments for these problems, and they are

keen to present the best and most beautiful image possible, so they take care of their skin, its freshness, the length of their hair, its luster and everything related to beauty, whether male or female [4].

With the passage of time and the improvement of the nature and conditions of human life, various types of cosmetics have appeared and become an essential part of the life of most people.

Cosmetics vary in terms of the types and purpose of use, and natural cosmetics manufactured from purely natural resources are among the best cosmetics and much better than other preparations with negative effects on the skin. Many people with allergies have found that natural products are the best for these matters, whether in oils, creams, or other.

The role of natural products is not limited to treatment only but includes everything related to the health and beauty of the body. There are natural essential oils for a beautiful body smell, and extracts to whiten the skin and unify its color, remove excess hair, lengthen the hair, smooth the feet, and many other benefits.

So natural and organic cosmetics are still growing due to the high demand from consumers who have been influenced by recent media and social media posts providing facts about green cosmetics, who produces them, how they are better for your skin than conventional cosmetics, the health risks of traditional colorants and so on. They also now discuss what is needed to create a product that falls into the category of eco-friendly products, and health-conscious consumers buy these products as a way to reduce their exposure to potentially harmful chemicals like parabens, while demanding that companies be transparent about the ingredients used in the products they use every day [5].

All these expectations and demands from consumers have put high pressure on manufacturers and business owners, who have started investing much money to press researchers and scientists to discover natural-source compounds for use in creating new effective products that attract consumers [6].

1.3.2. Structure of the study

Ajwa dates are one of the primary fruits used in this field because their chemical compounds have beneficial effects on the body, such as their anti-inflammatory, anti-bacterial and antioxidant effects. Therefore, this study aimed to find the best way to extract antioxidants from Ajwa dates and seeds.

Ajwa is one of the date palm types cultivated in the Medina region of western Saudi Arabia. It is considered the most expensive of all dates due to its sanctity for Muslims, because the Prophet Muhammad recommended eating Ajwa dates 1440 years ago. It has a high nutritional value because it contains a high percentage of sugar, dietary fiber, vitamins, and minerals. According to many studies, it has also been reported for antioxidant activity, which is attributed to compounds with free radical scavenging activity due to its phenolic content. The date meat part generally contains digestible sugars such as glucose and fructose, while the kernels are a rich source of dietary fiber, minerals, and bioactive components, but because there is less awareness of their benefits, a large number of seeds are wasted. Therefore, an experiment was designed to compare the antioxidant properties of Ajwa date fruit and seeds using three different extraction methods and to assess the ability for root scavenging using DPPH and ABTS assays and measurement of the phenolic components TPC and TFC.

The purpose of this study was to investigate the possibility of using Ajwa dates and their seeds as functional ingredients of cosmetics by comparing the results of root scavenging tests according to the extraction method.

II. Literature review

2.1. Review of research on date palm

2.1.1. Ingredients of date fruits and seeds

The date palm fruit (*Phoenix dactylifera L.*) is one of the most widely used fruits in the world, based on its valuable properties. Mainly, it is rich in dietary fiber and phenolic antioxidants [35]. The nutritional composition of date fruits has been well documented, as many researchers have reviewed the approximate composition of some date fruits in the full maturity stage. According to the researchers, the average protein content is 1.5%, fat 0.14%, ash 1.16%, and carbohydrates 54.9% in date fruits [36].

a. Carbohydrate

The essential carbohydrate components in date fruit are glucose, fructose,. They include maltose (33.7 mg%), fructose (22.8 mg%), and glucose (22.3 mg%), which are easily absorbed by the body to provide energy quickly [37]. Different carbohydrate concentrations of date fruit can be attributed to differences in the cultivar, harvest and post-harvest factors and growing environment. The total sugar concentration and the percentage of glucose and fructose in the date fruit also change with the growth stage, where the total sugar concentration usually increases from the chimeric stage to the date stage. The date variety can also significantly affect the glucose and fructose content in the fruit. Many researchers have observed that the total glucose/fructose ratio in (Khalas) was higher than the glucose concentration in the date stage in all the studied dates.

b. Fiber

Date fruit contains 6.5–11.5% dietary fiber, of which insoluble dietary fiber is 84–94% and the rest is 6–16% soluble dietary fiber. Date fruit seeds are a rich source of dietary fiber too, containing about 15% fiber and are characterized by a high

level of water-insoluble fiber. Insoluble dietary fiber (hemicellulose, cellulose, and lignin) is also the main component of the seed fiber. Dietary fiber has essential therapeutic effects. The fiber concentration also depends on the variety and stage of maturity. The total concentration of fiber decreases in the date stage [38,39].

c. Minerals

Date seeds contain many minerals such as potassium, copper, magnesium, calcium, chromium iron, manganese, zinc, nickel, cobalt, phosphorus, and lead. The potassium concentration is the highest among the minerals, reaching 27.2 g/kg [40]. The concentration of minerals in dates increases with maturity in some varieties, such as in the Degla Noor variety, and other researchers have found that the mineral concentration decreases with the ripening of the dates in five other varieties [41].

d. Vitamins

Date fruit has reasonable amounts of vitamins, including vitamin A, vitamin K, vitamin C, and folic acid. However, it is rich in vitamin B complexes, such as (B1), (B2), (B3), (B5), (B6), and (B9). The vitamin concentration in date fruit decreases with maturation owing to drying and environmental factors [42].

e. Protein

The protein concentration in the date fruit is about 3–10 g/kg, and it varies according to the date variety and the stage of maturity. Most of the proteins in date fruit are soluble zolamine. The protein concentration in the fruits of the date at the Kimry stage is 6%. After that, the protein concentration gradually decreases to 2.5% in the Tamar stage due to non-enzymatic browning reactions (Maillard) and tannin precipitation. Dates contain 23 amino acids, most of which are not found in the most popular types of fruits, such as glutamic acid, lysine, alanine, serine, aspartic acid, proline, and glycine [43].

f. Fat and fatty acids

The date fruit contains 0.2–0.5% oil. To date, knowledge of the fruit oil composition has been limited, but the common rich fatty acids are oleic palmitic acid and linoleic acid [43]. Also, the seeds contain 7.7–9.7% fatty acids, which represent a group of moist and unsaturated acids, including 14 types of fatty acids. Several studies have recorded that date seed oils contain high levels of oleic acid. The differences in date fruits range from 41.3% to 47.7%, but the highest oleic acid content (53.3–58.8%) was found in the date seed oil extracted from the UAE varieties. The fatty acid composition of date seed oil appears to differ slightly with the varieties [44]. It also has the advantage of being more yellow than other vegetable oils. Date seed oils can be preserved for a long time due to their high oxidation rate. Regarding this property, it can be used as a preservative in cosmetics [45]. Also, studies were conducted on the profiles of the extracted oleic fatty acids. In one study, gas and liquid chromatography was used to measure the content of saturated and unsaturated fats in dates. Their seeds showed that the primary unsaturated fatty acids were linoleic acid (32.77%) for date fruits and oleic acid (47.66%) for date fruit seeds. In contrast, the primary saturated fatty acid was palmitic acid (20.55%) for the date fruit and lauric acid (17.39%) for the seeds of the date fruit. Myristic, stearic, and linolenic acids are also found in pulp and seeds [46]. In one research work conducted on the extraction from date seeds to measure the antioxidant activities of oils from 15 types of Iranian dates, the seeds were examined to determine the value of the DPPH root scavenging of the oils' antioxidant activities. The results showed an activity of 30.1%. These results could encourage interest in a variety of date fruits and date seed oils for use as an ingredient in cosmetics due to the advantage of reducing the concentration of free radicals [47]. Oleic acid occupies the most significant proportion of oils extracted from dates and their seeds, from long-chain mono-unsaturated and poly-unsaturated fatty acids, and is naturally present in various animal and vegetable fats. Chemically, oleic acid is listed as an omega-9 mono-unsaturated fatty acid and abbreviated as C18: 1, the most common fatty acid in nature [48].

Table 1. Date fruit and seed nutritional values.

(* g/100 g; # mg/100 g)

Nutritional ingredients	Date fruit	Date seed
Carbohydrate*	65.7–88.02	2.43–4.65
Protein*	1.22–3.30	4.81–5.84
Dietary fiber*	1.9–16.95	67.56–74.20
Ash*	1.43–6.20	0.82–1.14
Fat*	0.11–7.33	5.71–8.77
Moisture*	9.43–21.53	8.64–12.25
Iron #	0.3–2.2	2.30–2.21
Sodium #	4.9–8.9	10.25–10.4
Potassium #	289.6–512	229–293
Phosphorus #	12–27	83.6–68.3
Calcium #	123–187	28.9–38.8
Magnesium #	56–150	51.7–58.4

2.1.2. Bioactive compounds in date fruit

Phenolic compounds, a large group of secondary plant metabolites, are a class of biologically active substances that contain a benzene hydroxyl ring with one or more carboxyl groups. Several studies have shown that the date fruit is rich in phenolic compounds [49], and they are one of the most prominent phytochemical groups because they show a diversity in the dominant structures in plants. They are a necessary part of the human diet and are essential due to their antioxidant properties [50]. Their structures range from simple phenolic molecules, like phenolic acids, to polyphenols like flavonoids, to high molecular weight compact polymers. In one study, researchers were working to identify and measure the phenolic composition of date seeds, and two analyses were performed on the seed type as follows: 1 - Analysis of simple phenolic compounds (phenolic acids, hydroxycinnamic acids, flavanols, flavonoids, flavan-3-ols) 2 - Analysis of all flavans (monomers, proanthocyanidin oligomers, and polymers) after depolymerization. The total amount of phenolic compounds was 2.19 ± 0.04 g/kg of date seeds before depolymerization. From the analysis of flavan-3-ols monomers and the constituent members of proanthocyanidins after depolymerization, it was reported that the number of flavan-3-ols was 350.18 ± 0.36 g/kg with epicatechin (46.80 ± 1.01) g/kg and catechin (3.38 ± 0.35) g/kg [51]. The complete phenolic contents of the various date fruits showed a remarkable difference. The differences in phenolic acid concentration in dates were due to the date variety, environmental conditions, etc. The highest percentages of phenol compounds were found in the Kimry stage, then Rutab, and Tamar was the lowest. Therefore, we can say that fresh dates are better in proportion to the presence of phenolic compounds than dried dates [52,53]. Many studies also found that the storage method and the type of solvent affect the level of measurement of the total phenolic content in the date fruit and the seeds. The highest level of phenolic compounds in some varieties of dates was found in the acetone extract, with 70%, and this level ranged from 199.43 to 576.48 mg/100 g fresh weight. However, extraction using 50% methanol gave fewer phenolic compounds but a higher antioxidant effect [54]. One study showed the effect of the solvent on the phenolic extraction from the date fruit, as well as of the temperature, the time of extraction, and the number of times of extraction [55].

Nevertheless, the data presented in one of the studies from the Kingdom of Saudi Arabia confirmed that the concentration of total phenolic compounds depends on the variety of dates and the extraction solvent as well, and the total phenolic compounds in a water extract (455.88 mg/100 g) is higher than the total phenolic compounds in alcohol extract. The comparison between water extract and alcohol was made on three types of dates. In the three types, the total phenolic compounds in the water extract were higher than in the alcoholic extract. The date fruit can be considered a rich source of hydrophilic antioxidants, and this reducing property is related to the presence of polyphenols, specifically flavanols [56]. Based on the results of a recent study of influences on the percentage of phenolic compounds, the effect of cooling the fruit before extraction was proven. The effect of cooling the fruit (at 4°C for 8 weeks) before extraction on the total phenolic content was evident in the result, as the total phenolic content of all the date fruit extracts increased after fruit cooling, except for methanolic extracts. However, the total phenol for acetone extracts increased by 50% to 217.86%. The ability of methanol to extract phenolic compounds from palm fruits was better than acetone, as revealed in various other results. The extraction performance of the phenolic compound by the strength of methanol and acetone also varies with different date palm species. In short, the type of solvent, the method of extraction, and the storage method are still subject to comprehensive and different information that needs wider studies on a higher level beyond the date palm species. The solvents used may extract not only phenolic compounds but also various compounds such as oxidizing or antioxidant enzymes, which may also be responsible for reducing or increasing the antioxidant activities of the methanol and acetone extracts [57].

The traditional extraction process using organic solvents is harmful and dangerous to humans and the environment. Therefore, researchers have studied the introduction of clean technology to extract compounds from date seeds. Based on the study results, supercritical carbon dioxide (SC-CO₂) is a uniquely suitable method for removing natural compounds because it does not require organic solvents. The highest oil yield was at 3.0% under operating conditions for an extraction pressure of 41.4 MPa at 70°C and 40 min, and the CO flow rate used was 24 mL/min with a molecular size of 0.5 mm.

This study will help extract a high-value natural compound for multiple applications, including the cosmetic industry [58]. There is no doubt that many types of research have been of great value in explaining the value of the palm tree, and many researchers have studied date palm cultivation, its value, and the extent of its use in therapeutic applications [59].

2.1.3. Date fruits and seeds in cosmetology

Damage to the outer skin develops due to several factors: extreme physical and psychological stress, poor nutrition, and exposure to UV rays. The generation of reactive oxygen species by ultraviolet rays in the skin leads to oxidative stress that causes free radicals [60]. Studies have provided compelling evidence for a strong association between free radicals and the development of premature aging [61]. It is estimated that UV rays contribute up to 80% of all environmental factors and are the most critical environmental factor in developing skin cancer and skin aging [62]. Some of the applied antioxidants can block the harmful effects of free radicals as a preventive measure, leading to the average production of the skin's structural proteins [61]. It is believed that the additional topical application of vitamins and antioxidants in cosmetics can better protect and possibly correct the damage by neutralizing the free radicals [63].

Free radicals are highly reactive molecules with an unpaired electron that damages the surrounding molecules and tissues, and it is known that ultraviolet light and environmental pollutants are among the initiators that cause free radicals. Because the skin is the external barrier in the human body, it is at the forefront of the battle with external influences to destroy free radicals [64]. It is believed that the antioxidant compounds in palm fruits and seeds, especially the phenolic compounds, play a protective role in humans and palms against environmental conditions, which may explain the high ability to adapt to the arid desert environment where palm trees grow [65]. The phenolic content and antioxidant capacity of dates from Middle Eastern countries have been studied extensively. Although there is little work on cosmetic formulations with date fruits and date seed derivatives, several authors suggest that they be incorporated into cosmetics based on their chemical composition and offer the ability to protect from ultra-violet rays [66]. Studies have shown that phenolic compounds contribute to fruit preservation by protecting against photo-oxidation and providing resistance against microbial and parasitic infections. This may explain the phenolic preservation function and why fruits of the same variety may exhibit different antioxidant capacities when grown in different geographical locations [67].

The presence of a phenol core acts as an effective sensor for reactive species, reducing ferric ions that stimulate lipid peroxidation. The beneficial effects of polyphenols have become essential to the cosmetic industry in terms of developing ecological products rich in green raw materials, especially for therapeutic products, and preventing premature skin aging [61]. One article describes the feasibility of using a cosmetic cream with an aqueous extract and oil from the seeds of date fruits. Three results were taken into account: i) expandability, ii) viscosity, iii) peroxide index. The results showed a relatively wide formulation range to ensure an adequate cosmetic cream with spread ability and viscosity. Date seed oil was found to have a positive effect on both the texture and peroxide index. It would be interesting to deepen some of the analyses (statistics and dermatological tests) to realize the potential benefits of the natural ingredients in the cosmetic cream and the potential of dates in particular [68].

There are some potential applications of date seed oil in cosmetics (body creams, soaps, hair products, and sunscreens). Date seed oil has high oxidative stability and can be stored safely for a long time [69]. It can protect the skin from the UV-A and UV-B rays responsible for cell damage and from oxidative stress damage caused by hydrogen peroxide, which gives the ability to intervene in different stages of the oxidation mechanism. Additionally, date seed oil can repair human skin due to its antioxidant activity. These properties make it suitable for the cosmetic industry [70]. Date palm possesses anti-wrinkle properties due to phytohormones that reduce wrinkle surface area and depth and significantly improve skin elasticity. This research has proven the effectiveness of the date palm cream on volunteers after eight weeks of application [63]. The presence of vitamins such as ascorbic acids and vitamin E in date extracts helps to improve the moisture level in the skin by stimulating dermal fibroblasts and increasing collagen production. Moreover, the presence of minerals and vitamin C may be linked to the anti-aging ability of dates [71].

The most common micronutrients with dermatological results are vitamin B1, C, A, E and K, Fat-soluble, riboflavin, niacin, pyridoxine, biotin.

The minerals zinc, iron, copper, and selenium.

Essential fatty acids and these nutrients were mentioned earlier, and they were confirmed in date extract and date seeds, so the use of these nutrients in cosmetic ingredients extracted from date fruit helps protect the environment and protect these components from damage and invest them in human health [72]. Several studies describe hair diseases of three types: 1. alopecia, meaning hair loss, 2. early graying of hair, and 3. baldness. There are many plants and extracts used in different parts of the world for hair care that have an activity that promotes hair growth, including hair conditioner, hair cleansing agent, anti-dandruff agents, and the treatment of hair loss [73]. However, there is no scientific study applied to the use of date extracts and their seeds. On hair and explaining the benefits of date extracts on hair, therefore, in this review, studies of different plants will be mentioned. The benefits of phenolics and procyanidin flavonoids for hair have been proven, and these compounds have been found to be present in date extracts and their seeds [74]. The relationship between flavonoids and phenolic substances has been reported in treating hair loss and hair growth-promoting activity. Experimental studies have shown that the phenolic substances in several plant extracts appear to be potent inhibitors of the 5-reductase enzyme, which is responsible for converting testosterone into DHT, the alleged cause of hair loss in men [61]. Flavonoids helped increase the length of hair and the growth phase in the hair follicles of mice, because the formula contains an excellent activity to promote hair growth. It works mainly by enlarging the size of the follicle and prolonging the stage of development [75]. It is reported that the phenolic substances in green tea affect hair loss, and I concluded that the anti-inflammatory and anti-stress effects of these natural substances might affect the growth of human hair, and date extracts are rich in these substances [76].

In an experimental study conducted on human hair, images showed that applying an-

tioxidants to the hair fiber leads to an improvement in the mechanical properties of the hair. Because of the low molecular weight peptides penetrating the hair and improving the mechanical properties at the cortex level, the higher molecular weight peptides may form a protective layer that prevents degradation, tryptophan and loss of color. Moreover, lipid peroxidation and protein degradation in the hair is significantly reduced in the samples treated with antioxidants, indicating improved fiber integrity. The anti-oxidants are due to the hydroxycinnamic derivatives present in date extract and their seeds in good proportions [77]. A study showed that the formula of a hair serum that contains freeze-dried coconut water extract and flavonoids increases the hair density and hair growth in male and female volunteers suffering hair loss and helps prevent itching and sensitivity, so it is light, safe, and gentle on the scalp [78].

Vitamin B is recommended for strengthening hair and nails and is found in many cosmetics and health products for hair. Dates and seed extracts are good sources of vitamin B [79]. A recent study indicates that date seed oil extract is rich in the phytosterols, essential fatty acids, and nutrients needed to maintain a healthy scalp, promote average hair growth, and support nutritional functions of sebaceous glands and hair follicles [80]. It should also be mentioned that vitamin C and vitamin E have been reported in the fruits and seeds of the date palm. Vitamin E promotes the growth of scalp skin cells and acts as an ideal hair conditioner. As for the benefits for nails, vitamin E is characterized by its ability to protect the nails from aging or turning yellow, so it is an effective solution to all nail problems to moisturize and give a distinctive healthy appearance [81]. As for vitamin C, which is known for its importance as an essential ingredient in hair and nail care products, it helps reduce the formation of the free radicals that damage hair as well as nails. It helps maintain healthy nails by strengthening the skin, connective tissues, and walls of blood vessels [82].

The extract and seeds of dates have antioxidant capabilities of great importance because of their richness in polyphenols and tocopherols. Moreover, because they have a high degree of oxidative stability, better than most vegetable oils, they can be used as a dietary supplement

ment that not only aims to prevent changes within the skin caused by environmental stimuli, but is also increasingly applied to enable the skin to restore its natural balance after disturbances as quickly as possible, although the role and potential of cosmetic dermatology in repair processes at the cellular and molecular level still needs to be assessed in more detail [83].

Many cosmetics contain synthetic chemicals that adversely interact with the skin. Some common hazardous chemicals are commonly found in cosmetics. Paraben is the most common ingredient in most cosmetics and is used as a cosmetic preservative. Many consumers prefer paraben-free products because parabens are highly toxic and can cause allergic reactions and rashes on sensitive skin. So we can replace it with the natural preservatives in date seed oil [84].

A study reported the use of date syrup biomass waste. To manufacture soaps with enhanced bactericidal and antioxidant activity, they found that an assessment of antioxidants in soaps modified with date syrup extract showed an optimal extract concentration. The inclusion of this optimal date syrup extract concentration decreased the pH and moisture content, increasing the foam height and total fatty matter of the resulting soaps.

The physicochemical characteristics of soaps including date syrup extract were comparable to those of commercial turmeric soap. Furthermore, antibacterial assays demonstrated the enhanced bactericidal properties of date syrup extract soaps against gram-positive *Streptococcus pyogenes* and gram-negative *Pseudomonas aeruginosa* bacteria. Overall, the results support the use of date syrup extract as an economical and effective natural antioxidant and antibacterial agent for soap preparation [85].

The oil part of the date seeds can also be used with different health benefits because it contains ingredients with different biological effects, such as antivirals, anti-inflammatory, and some other activities that can be used in the manufacture of cosmetics and pharmaceutical care products for acne or wound healing [86]. On fatty acids, the content of myristic acid and oleic acid in date seed oil can be a good source for improving transdermal absorption by increasing the diffusion of non-lipophilic substances.

It becomes clear to us in each study that date extract contains many ingredients that can be used in various industries. One of those studies also proved that date extract contains fluorine, which helps protect the teeth from decay [87].

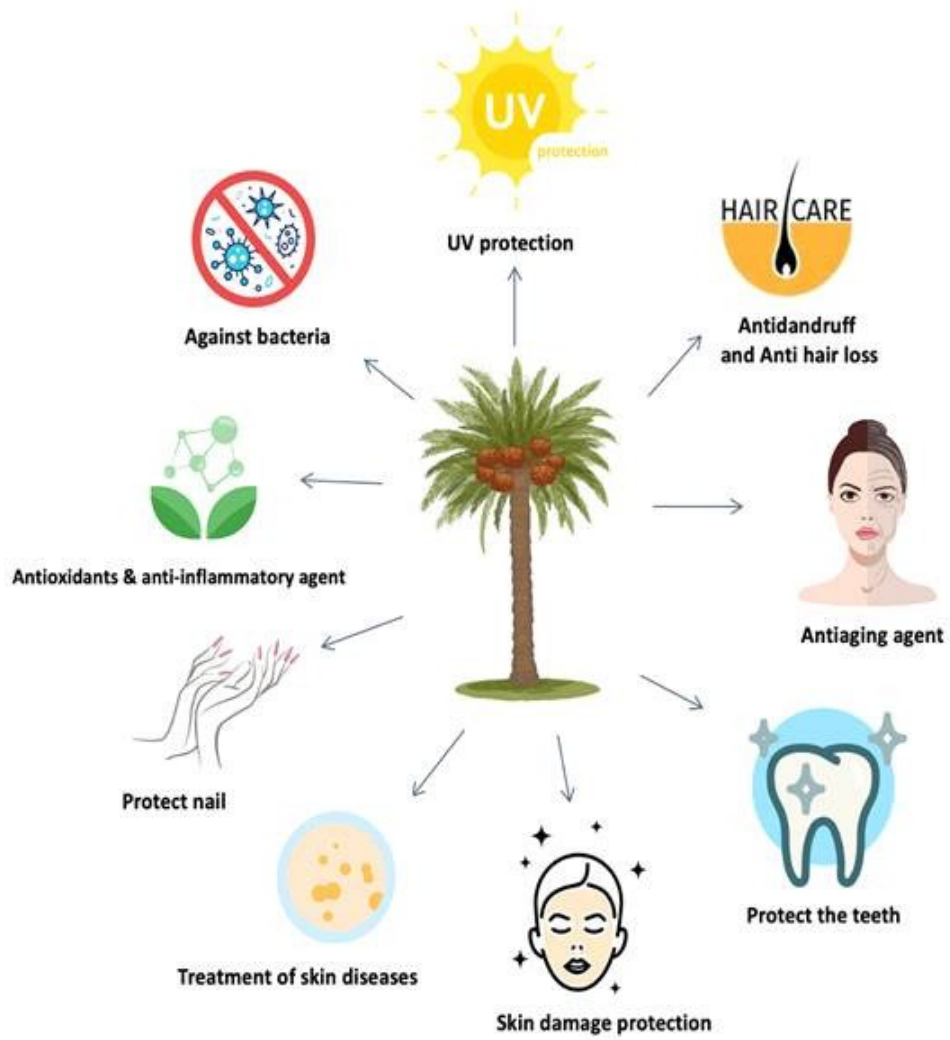
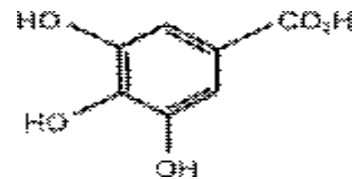
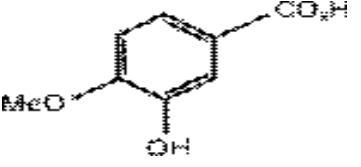
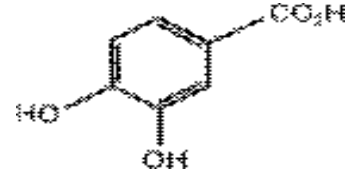
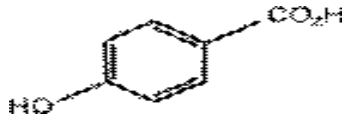


Figure 5. Instances of the cosmetic applications of date fruits and seeds.

Table 2. Chemical composition of date fruits and seeds and their use as cosmetic ingredients.

Compound name	Chemical structure	Proof of efficacy as an ingredient in cosmetics	Ref.
Gallic acid		Gallic acid is thermally fluctuating, making its effectiveness as a cosmetic ingredient challenging. To overcome this flaw, gallic acid was linked with a peptide, creating Galloyl-RGD, which is a promising candidate as a cosmetic ingredient.	[46][74]
Vanillic acid		Vanillic acid contributes to lightening the skin and reducing pigmentation on human skin. Its penetration into the skin has been confirmed, and the toxicity test has been passed, increasing its application on the skin.	[47][75]
Protocatechuic acid po-		Study results on human skin show protocatechuic acid's potential in skin aging treatments in 8 weeks.	[48][76]
p-Hydroxybenzoic acid		p-Hydroxybenzoic acid is used in cosmetic and personal care products to contain the growth of microorganisms and, therefore, lengthen product shelf life.	[49][77]

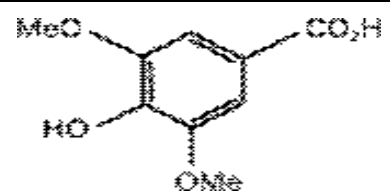
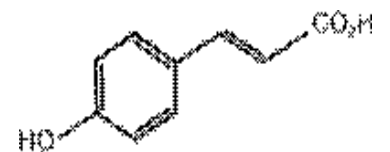
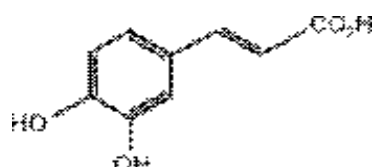
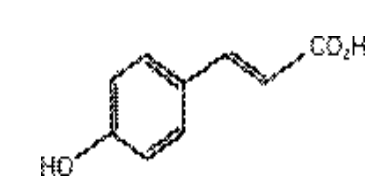
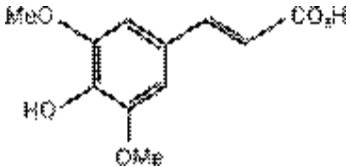
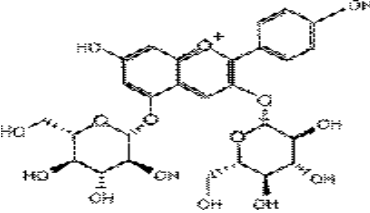
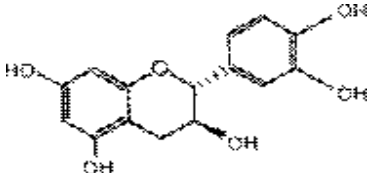
Compound name	Chemical structure	Proof of efficacy as an ingredient in cosmetics	Ref.
Syringic acid		Syringic acid helps stop cell aging due to ultraviolet B being defeated. Consequently, the antioxidant and antiaging syringic acid results improved the survival rate of cells destroyed by ultraviolet B, implying that it can be applied as a natural phytochemical in cosmetic products.	[50][78]
Cinnamic acid		Cinnamic acid derivatives are widely used in cosmetic products such as UV protection, antioxidant and antimicrobial agents.	[51][79]
Caffeic acid		Caffeic acid has gained remarkable attention as promising and safe from free radical toxicity. Because of this, it is present in cosmetic products for its antioxidant activity.	[52][80]
Ferulic acid		Ferulic acid is an inhibitor of proteins that catalyzes free radical formation and enhances scavenger protein activity. Ferulic acid has a protecting role for the central skin structures. It frustrates melanogenesis, improves angiogenesis, and stimulates wound healing. It is generally used in skincare formulations as a skin photoaging brightening ingredient.	[53][81]

Table 2. (Continued)

Compound name	Chemical structure	Proof of efficacy as an ingredient in cosmetics	Ref.
Sinapic acid		Sinapic acid saves the skin cell from total collagen degradation by inhibiting UVB activation, weakening photoaging-associated changes in vivo, and stopping the inflammation of the skin tissue.	[54][82]
Pelargonic acid		Pelargonic acid is a fatty acid that can function as a fragrance ingredient and surfactant-emulsifying agent. In cosmetics, it can function as a surfactant-cleansing tool. The Cosmetic Ingredient Review (CIR) Expert Panel previously decided that this ingredient is secure in cosmetic products to enhance skin penetration.	[55][83]
Catechin		Catechin can increase collagen arrangement by crosslinking. Studies confirm that collagen fibers bind with catechin molecules.	[56][84]

2.2. A review of research on Ajwa dates

Ajwa is an excellent fruit; it is a medicine and has preventive qualities. Based on this, many researchers from various fields of health sciences have devoted efforts to discussing the best ways to benefit from this fruit to prevent and treat multiple diseases. Ajwa is a fruit of high nutritional and medicinal value and has promising therapeutic results that have been proven by many types of research, experiments, and applications.

a. Toxin prevention

Research conducted in Al-Madinah Al-Munawara on the date palm proved that it protects the organs from toxins. When conducting an experiment on rabbits, giving them lead acetate and monitoring the impact of essential organs such as the liver, heart, lung, and kidneys, and measuring the levels of lead in the blood, it was found that the rabbits that took the dates were little affected by lead acetate, unlike the rabbits that did not take Ajwa [88, 89].

b. Cell protection

The cells in the body undergo processes of demolition and restoration of molecules and organelles, especially the genetic material, which is responsible for the vitality of cells and tissues, because oxidative stress, which is a natural consequence of the body's metabolism causes the formation of free radicals within cells that cause damage to cells and tissues. Antioxidants are chemicals that inhibit free radicals and prevent them from causing damage inside the body and reduce the possibility of infection with many diseases. Several types of research have proven the effectiveness of Ajwa date, fruit or seed, as an antioxidant.

The results also showed that 30% of Ajwa extracts had a greater effect on lowering the lipoprotein profile, total triglycerides, total cholesterol, and LDL-C levels, in addition to a significant increase in HDL-C levels in treated white mice. This confirms that the dates' contents are polyphenols and antioxidants, have a beneficial health effect on the lip-

oprotein profile and can be used as an active ingredient against various diseases in nutritional products and supplements [90].

c. Anti-inflammatory

It has been proven in the laboratory that Ajwa dates are anti-inflammatory and antioxidant. The enzyme cyclooxygenase, especially 2-COX, produces inflammatory substances in the body and participates in genetic regulation. Therefore, inhibiting this enzyme offers a glimmer of hope in reducing inflammation and curing many diseases, and it has been proven that Ajwa dates work to inhibit this enzyme [91].

d. Inhibition of cancer cells

The results showed that the methanolic extracts of Saudi dates, including Ajwa, have an activity to inhibit cancer cells in the stomach, prostate, colon, breast, and lung [92].

III. Materials and methods

3.1. Experimental materials and reagents

3.1.1. Materials

The devices used are a sensitive scale, Autoclave, LM2 mill, filter paper (Whatman No. 1), Rotarapor, electromagnetic, vibrating heater, Elisa reader, freeze dryer, different glassware (cups, conical flasks, standard flasks of 500 ml capacity), funnels, graduated condensers, glass stem, condenser, thermometer and distilled water

3.1.2. Reagents

Reagents used for extraction in this study were ethanol (DUKSAN), methanol (DUKSAN), and ethyl acetate (DUKSAN). For various physiological activity tests, 1,1-Diphenyl-2-picrylhydrazyl (DPPH, SIGMA), L-ascorbic acid (SIGMA), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS, SIGMA), folin-ciocalteus phenol reagent (SIGMA), potassium persulfate (SIGMA), KCl (DUKSAN), NaCl (DUKSAN), NaCO₃ (DUKSAN), Na₂HPO₄ (DUKSAN), KH₂PO₄ (DUKSAN), gallic acid (SIGMA), aluminum chloride (SIGMA), potassium acetate (SIGMA), quercetin (SIGMA), kaempferol (SIGMA), isorhamnetin (SIGMA), quercetin dihydrate (WAKO), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, SIGMA), methanol (THERMO FISHER SCIENTIFIC), phosphoric acid (DUKSAN) and ethanol (THERMO FISHER SCIENTIFIC) were used. All reagents used for HPLC analysis were HPLC grade reagents.

3.2. Extraction and isolation

3.2.1. Sample collection

Ajwa fruit samples were procured from a farm located in the Madinah region. This farm cultivates only Ajwa date palms. 4 kg of fruits were collected and shipped to Gwangju Korea Chosun University. At the time of harvest, the fruit was in the "Tamar" or ripened stage.

3.2.2. Extraction

The seed was separated from the date, and the fruits were cut into slices, then placed in a glass container: three 250g samples with 100% ethanol solvent for seven weeks, two 50 g samples in 70% ethanol solvent for 1 week, and one 50 g sample with 500 ml hot water for 2 hours using the autoclave. Moreover, the solvents were used in the same ways with the date fruit, after crushing the seeds in an LM2 mill.

The six extracts were filtered through filter paper (Whatman No. 1, Whatman International Ltd., Maidstone, UK), the solvent was separated using a Rotarapor and the samples were dried with a Centea-vac.

Finally, a freeze dryer MLU was used.

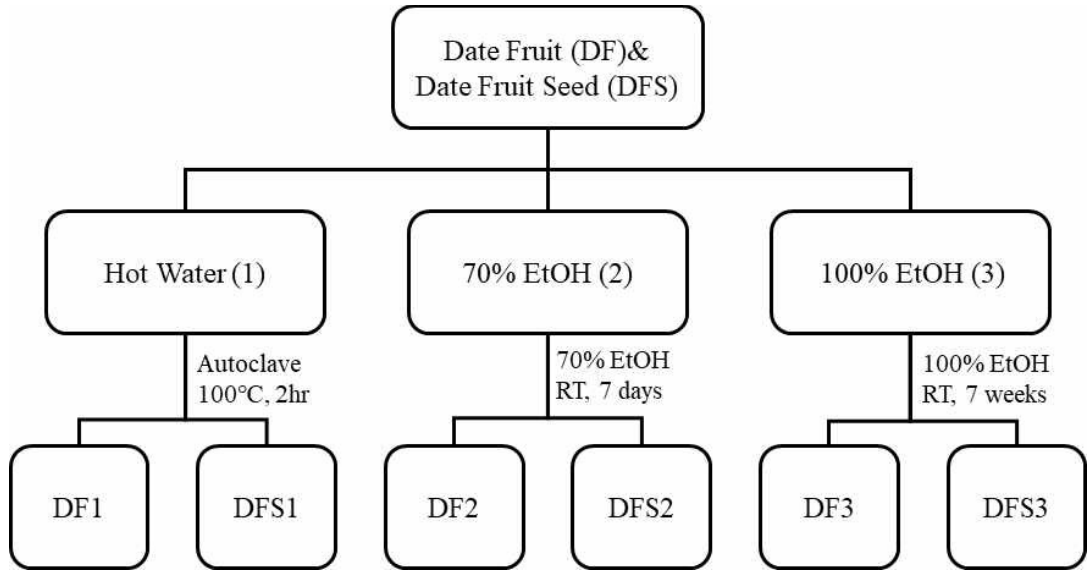


Figure 6. Isolation diagram of date fruit (DF) and date fruit seed (DFS).

3.3. Antioxidant activity

3.3.1. DPPH free radical scavenging assay

The DPPH radical scavenging ability was measured by modifying the method of Khaled [93]. 800 μL of 0.5 mM DPPH reagent was mixed with 200 μL of each concentration of date fruit and seed extract solution, and after reacting in the dark for 30 minutes, Biotek absorbance was measured at 517 nm wavelength using the Synergy HT multi-detection microplate reader equipment. A comparative experiment was conducted using gallic acid as a positive control group. The radical scavenging ability was calculated by the following equation and expressed as a percentage. Each reaction was measured in triplicate:

$$\% \text{ of scavenging activity} = (A \text{ control} - A \text{ sample}) / A \text{ control} \times 100$$

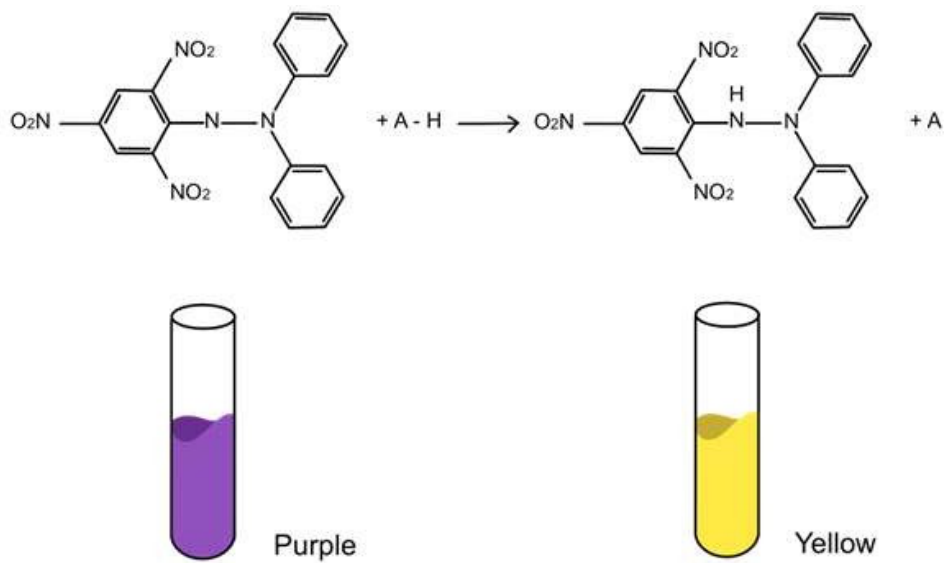


Figure 7. Measurement of the activity of an antioxidant by the DPPH assay.

3.3.2. ABTS radical scavenging assay

The ABTS radical scavenging ability was measured by modifying the method of Biglari et al. [94]. 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 14 mM) was prepared at a concentration of 7 mM and potassium persulfate (4.9 mM) at a concentration of 2.45 mM after mixing in a ratio of 1:1. This solution was diluted with phosphate-buffered saline (0.1 M, pH 7.4), so the absorbance value at 730 nm could be 0.9 ± 0.02 . After mixing 1000 μL of ABTS solution with 200 μL of extracts of date fruit and seed extracts for each concentration and reacting in the dark for 15 minutes, the absorbance was measured at 730 nm wavelength using the Biotek Synergy HT multi-detection microplate reader equipment. A comparative experiment was performed using quercetin as a positive control. The radical scavenging ability was calculated by the following formula and expressed as a percentage. Each reaction was measured in triplicate:

$$\% \text{ of scavenging activity} = (A \text{ control} - A \text{ sample}) / A \text{ control} \times 100.$$

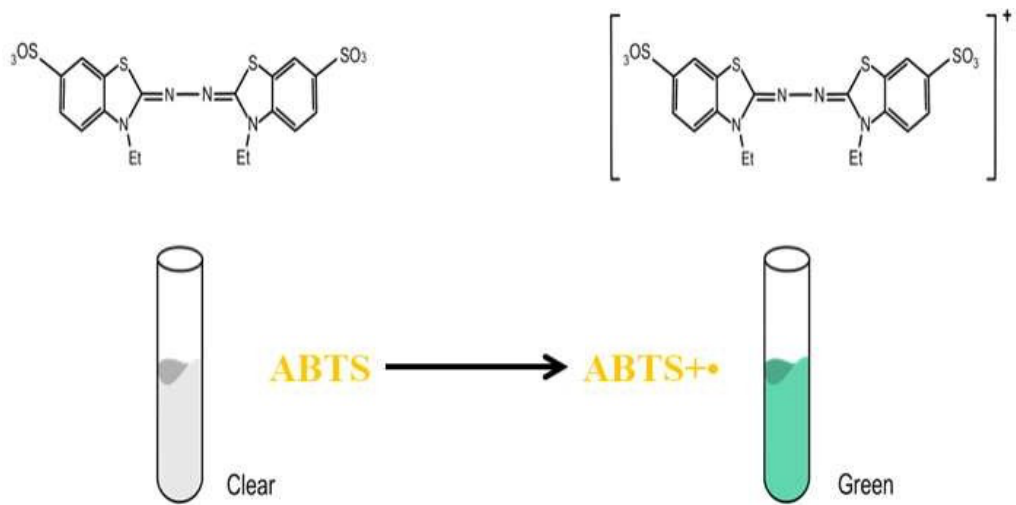


Figure 8. Measurement of the activity of an antioxidant by the ABTS assay.

3.4. Total polyphenol and flavonoid contents

3.4.1. Determination of total polyphenol content

Total polyphenol content and flavonoids were measured by modifying the method of Arshad et al. [95]. 500 μL of 0.2 M Folin-Ciocalteu's phenol reagent and 500 μL of 2% sodium carbonate aqueous solution (w/v) were mixed with 500 μL of extract solution of date fruit and seed for each concentration and reacted in the dark for 30 minutes.

Absorbance was measured at 750 nm wavelength using the Biotek Synergy HT multi-detection microplate reader equipment. The final concentration of the extract was 500 $\mu\text{g}/\text{mL}$, and the total polyphenol content was expressed as gallic acid (GAE) and p-coumaric acid mg/g equivalent based on the calibration curve.

3.4.2. Determination of total flavonoid content

Total flavonoid content was measured by modifying the method of Arshad et al. [95]. After adding methanol 1.5 mL, 10% aluminum chloride 100 μ L, 1 M potassium acetate

100 μ L, and distilled water 2.8 mL to 500 μ L of extract solution of date fruit and seed for each concentration, it was reacted at room temperature for 40 minutes.

Then Biotek Synergy HT absorbance was measured at a wavelength of 415 nm using a multi-detection microplate reader. The final concentration of the extract was 500 μ g/mL, and the total flavonoid content was expressed as quercetin (QUE) and rutin mg/g equivalent based on a calibration curve.

3.5. Analysis of polyphenol compounds

3.5.1. High-performance liquid chromatography (HPLC)

The hot water, 70% EtOH and 100% EtOH extract of date fruit and seed extracts were quantitatively analyzed by high-performance liquid chromatography (HPLC). The Shimadzu HPLC system (Prominence Modular HPLC, Kyoto, Japan) consisted of a LC-20AD pump, a diode array detector (SPD-20A), and a Shim-pack GIS-ODS C18 column (4.6×250 mm, >5 μM) (Table 3). The mobile phase consisted of water with % (v/v) formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B).

The flow rate was kept at 0.5 mL/min. The gradient program was as follows: 90% A/10% B, 10 min; 35% A/65% B, 60 min. The injection volume was 20 μL, and peaks were monitored at 280 nm. Peaks were identified by congruent retention times compared with standards (gallic acid, chlorogenic acid, catechin, vanillic acid, rutin, p-coumaric acid, ferulic acid, quercetin).

Table 3. Analysis conditions of high-performance liquid chromatography for polyphenol analysis.

Analysis condition	Explanation
HPLC equipment	Shimadzu LC-20 series
Solvent	- Solvent A: water (in 0.1% formic acid (v/v)) - Solvent B: acetonitrile (in 0.1% formic acid (v/v))
Mobile phase	90% A/10% B, 10 min; 35% A/65% B, 60 min.
Flow rate	0.5 mL/min
Column	Shim-pack GIS-ODS C18 column (4.6×250 mm, > 5 μM)
Oven temperature	RT
UV wavelength	280 nm
Injection volume	20 μL
Total run time	60 min

3.6. Cosmetic manufacturing and measurement of cosmetic properties

3.6.1. Cosmetic manufacturing

A cream containing date fruit extract was prepared in O/W formulation by the following process. In a beaker, date fruit extract water, 1,2-hexanediol, glycerin, and panthenol were added, which are water-based ingredients, in the proportions shown in Table 4, and melted on a hot plate at 70°C until a uniform phase was formed. In another beaker: Butyrospermum Parkii (Shea) Butter; Oenothera Oiennis (Evening Primrose) Oil, Cetearyl Alcohol/Cetearyl Oliviate/Sorbitan; and Oliviate/Polyglyceryl-3 Methylglucose Distearate/Glyceryl Stearate/Hydrogenated Lecithin/Stearic Acid/Ceramide NP/Butyrospermum Parkii (Shea) Butter were added in the proportions shown in Table 4, and melted on a hot plate at 70°C until it became a uniform phase. When the contents were completely dissolved in the two beakers, the oily material was slowly mixed with the aqueous material and stirred with a homo mixer (Mark II model, T.K. Primix, Japan) for 5 min. After stirring, when the temperature was lowered to about 40–50°C, date fruit extract and Citrus Aurantium Bergamia (Bergamot) leaf oil were added and stirred for 5 minutes. After defoaming the prepared cosmetic formulation, it was cooled slowly at room temperature to complete the formulation.

3.6.2. pH measurement

The pH of the cosmetic formulation was measured using the Orion Star™ A211 pH Benchtop Meter from THERMO SCIENTIFIC.

3.6.3. Viscosity measurement

Viscosity was measured using Brookfield's DV2T model. After placing 40 g of the cosmetic formulation in a 50 mL conical tube and immersing the spindle (Spindle, RV-06) in the formulation, the viscosity value was measured while increasing the rotor speed from 2 rpm to 100 rpm. The viscosity values were read after rotating for 120 seconds at 12 rpm and 30 seconds each at the other speeds.

Table 4. Cosmetics formulation with date fruit & seed extract.

Phase	Component	
Water phase	Date fruit extract water	63.4
	1,2-Hexanediol	2
	Glycerin	3
	Panthenol	0.3
Oil Phase	Butyrospermum Parkii (Shea) Butter	20
	Oenothera Biennis (Evening Primrose) Oil	5
	Cetearyl Alcohol/Cetearyl Olivat/Sorbitan	
	Olivate/Polyglyceryl-3 Methylglucose Distearate/Glyceryl	5
	Stearate/Hydrogenated Lecithin/Stearic Acid/Ceramide	
	NP/Butyrospermum Parkii (Shea) Butter	
Additive	Date fruit extract	1
	Citrus Aurantium Bergamia (Bergamot) Leaf Oil	0.3
	Total	100

IV. Results and discussion

4.1. Yield of extract according to extraction method

The extraction of natural products also shows differences in the extraction yield because the components differ according to the solvents used and the method of extraction. In this experiment, the fruits and seeds were extracted by three methods

Extraction yield was obtained by the formula fruit and seed extracts.

$$\text{Yield (\%)} = \frac{\text{Weight of concentrated sample after extraction (g)}}{\text{Weight of dried date fruit and seed (g)}} \times 100$$

Table 5 summarizes the yield of each extract according to each extraction method for the extracted fruits and seeds, confirming that the content of the seed extract was higher than that of the fruit extract. It was found that the yield of seed and fruit extract was highest at 11.24% and 5.24%, respectively, when using 100% EtOH and the yield of seed and fruit extract extracted using hot water was 3.95% and 7.15%, respectively, showing the lowest return.

Table 5 . Extraction yields of Date fruit and seed extracts

Sample	Weight (g)	Yield (%)
DF1	9.14	3.65
DF2	12.45	4.98
DF3	13.10	5.24
DFS1	3,78	7.56
DFS2	3,23	6.46
DFS3	3,37	11.24

4.2. Results of antioxidant activity

4.2.1. DPPH free radical scavenging activity

Measuring the radical scavenging activity of DPPH is a widely used experimental method because the antioxidant activity of natural products can be easily measured with DPPH, a relatively stable free radical, and it is closely related to the actual antioxidant activity. In this experiment, extracts of fruits and seeds were prepared at a concentration of 100–1000 $\mu\text{g/ml}$ to confirm the radical scavenging capacity of DPPH and the measured IC50 values. The results are shown in Figure 9. As a result of the measurement for each part, it was confirmed that there was higher activity in the seed extract compared to the fruit extract. The 70% ethanol extract for seed (DFS2) showed the highest scavenging activity of 92.25%, and was higher than the rest of the extracts. The extract with hot water also showed low scavenging activity in the seeds but high scavenging activity in the fruit (DF1).

In a study conducted by Khaled [93], it was reported that the DPPH scavenging activity of the extract of dates and Ajwa seeds was superior to the fruit in the test.

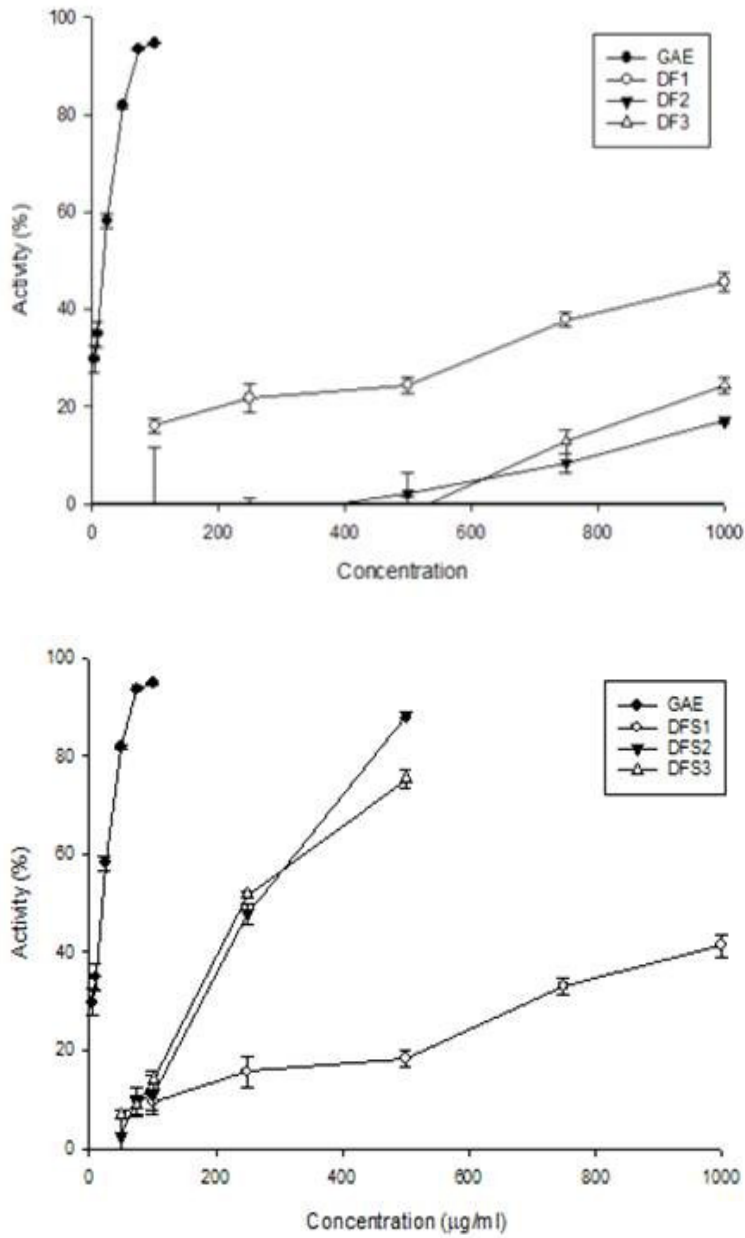


Figure 9. DPPH free radical scavenging activity results of date fruit (DF) and seed (DFS) extracts.

4.2.2. ABTS radical scavenging activity

The root scavenging capacity of ABTS was confirmed by preparing fruit and seed extracts at a concentration of 100–1000 $\mu\text{g/ml}$, and the IC_{50} values were measured. The results are shown in Figure 10, which indicate the highest scavenging activity in the 70% ethanol extracts of the seeds, with a scavenging activity of 97.41%, while for fruit the highest was in the hot water extraction, which showed a scavenging activity of 67.41%.

In a study by Khaled (2017), the ABTS radical scavenging activity of Ajwa date extracts was measured. Ajwa seeds have the highest amount of ABTS scavenging activity, in the range of 86.2% to 69.3%, followed by Ajwa fruits, which have ABTS scavenging activity varying from 65.9% to 54.2%, and this is considered a difference similar to this study of extraction methods.

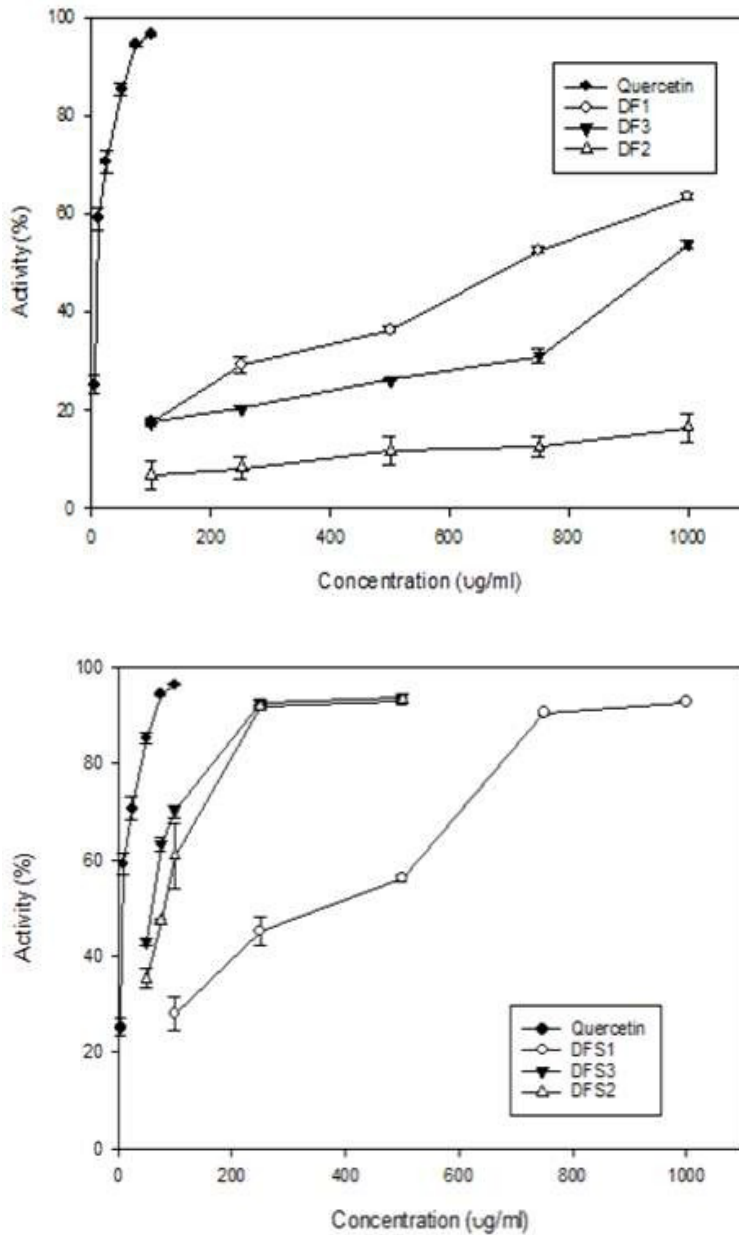


Figure 10. ABTS radical scavenging activity results of date fruit (DF) and seed (DFS) extracts.

4.3. Results of total polyphenol and flavonoid contents

4.3.1. Total polyphenol contents (TPC)

Polyphenol compounds are one of the secondary metabolites widely distributed in the plant kingdom and have different structures and molecular weights. Moreover, they bind easily to proteins and other large molecules due to the phenol hydroxyl group (OH) and are usually used as antioxidants and anticancer agents. Therefore, in this study, the total polyphenol content of fruit and seed extracts was measured, and the results are shown in Table 3. As a result of the measurement for each extract, it was confirmed that the polyphenol content in the hot water extract for the date fruit (DF1), was the highest, and reached 3008.66 $\mu\text{g/g}$, while for date fruit seed the 100% EthO extract (DFS3) reached 2516.35 $\mu\text{g/g}$ in Saleh's study [96], proving that the total polyphenols concentration was the highest in Ajwa date fruit in that study comparing three types of date fruit from Saudi Arabia.

4.3.2. Total flavonoid contents (TFC)

Flavonoids are polyphenol compounds, usually including catechin, quercetin, and rutin, the most potent antioxidants. They are found in different parts of plants such as the stems, leaves, roots, flowers, and fruits. In this study, the total flavonoid content of Ajwa seed and fruit extracts was measured, and the results are shown in Table 3. It also reveals that there are vast differences in flavonoid contents, with the highest content in 100% ethanol extract of seeds (DFS3). As for the fruit, the hot water extract had the highest content of flavonoids (DF1). In Khalid's study (2017), the amount of TFC ranged from 1897.4 mg to 2956.2 mg, in solvent (80% acetone). The results also indicate that 80% acetone was the best solvent for extracting flavonoids from both parts (fruit and seeds). The significant effect of the solvent and its different concentrations is also evident in the flavonoids, These differences may be due to the changing polarity of the solvents. that play an essential role in enhancing the solubility of flavonoids [93].

4.4. Analysis of polyphenol compounds

4.4.1. Results of HPLC chromatogram

HPLC analysis was performed to confirm the polyphenols present in the extracts of Ajwa date fruits and seeds, and the results are shown in Figure 11 and Table 6. Gallic acid, chlorogenic acid, catechin, vanillic acid, rutin, p-coumaric acid, ferulic acid, and quercetin were used as standard materials. As a result of HPLC analysis, it was confirmed that the standard content of polyphenols was highest in the hot water extract of seeds and fruits, and the content of gallic acid was much higher in the DF1 extract. These results are inconsistent with the antioxidant activity and total polyphenol content for fruit, which suggests that polyphenols not included in standard substances are included in Ajwa date extracts, considering the unknown extract peak. In addition, their activity is highly dependent on the phenolic compounds in the sample, but it is assumed that other unknown substances are also included.

However, the seed extracts results are consistent with the antioxidant activity and total polyphenol content. In Abdul-Hamid's (2019) study, the results showed that there are greater amounts of many metabolites in date seeds compared to date fruits [97].

Table 6. Phenolic compounds identified in date fruit quantified by HPLC.
(unit: $\mu\text{g/g}$)

No.	Standard	DF1	DF2	DF3	DFS1	DFS2	DFS3
1	Gallic acid	2,706.2	8.51	2.98	215.21	100.57	145.69
		± 10.22	± 2.14	± 0.97	± 1.32	± 3.47	± 2.08
2	Chlorogenic acid	190.81	4.60	33.53	479.56	170.07	284.08
		± 8.31	± 1.07	± 4.58	± 1.05	± 1.95	± 3.37
3	Catechin	64.35	1.87	6.52	981.33	139.99	314.58
		± 4.69	± 0.88	± 1.34	± 3.41	± 1.02	± 3.48
4	Vanillic acid	17.48	1.61	0.64	312.66	178.91	188.02
		± 3.59	± 0.24	± 0.09	± 2.28	± 2.01	± 2.13
5	Rutin	25.84	9.36	30.17	390.92	76.09	67.92
		± 2.68	± 1.37	± 3.51	± 1.01	± 1.10	± 1.16
6	<i>p</i> -Coumaric acid	3.96	10.09	10.97	122.31	42.17	39.00
		± 1.15	± 2.77	± 2.33	± 5.85	± 1.76	± 1.01
7	Ferulic acid	-	± 0.24	± 1.25	± 1.84	± 0.63	± 0.68
					5.06	1.87	3.67
8	Quercetin	-	-	-	± 1.15	± 0.99	± 1.81
	Total	3008.66	38.03	92.16	2516.35	713.74	1046.72

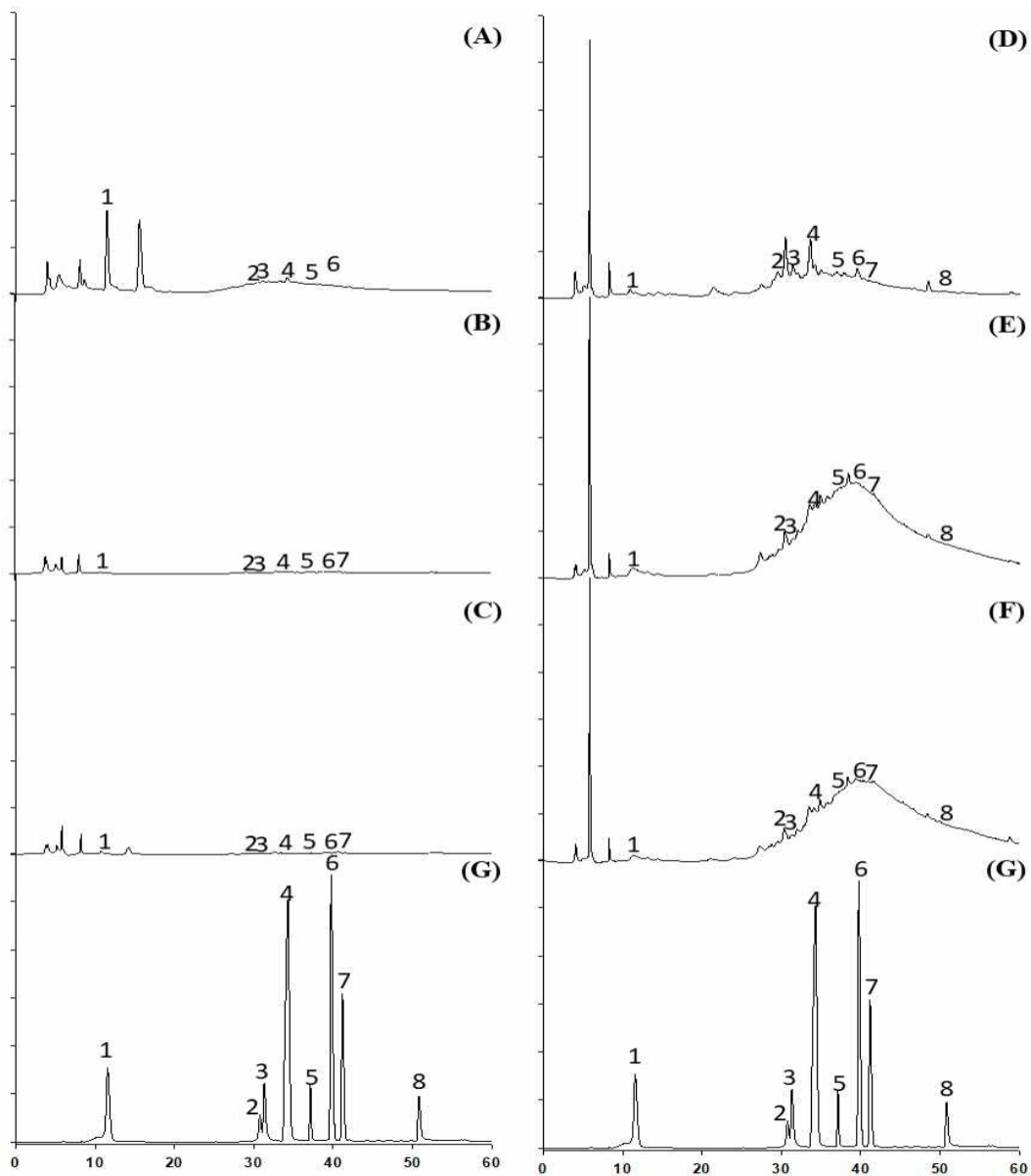


Figure 11. HPLC chromatogram of the date fruits (DF) and seed (DFS) extracts and standard mixture using diode array detection at 280 nm. (A) DF1; (B) DF2; (C) DF3; (C) DFS1; (E) DFS2; (F) DFS3; (G) standard mixture. Numbers indicate the following: (1) gallic acid; (2) chlorogenic acid; (3) catechin; (4) vanillic acid; (5) rutin; (6)p-coumaric acid; (7) ferulic acid; (8) quercetin.

4.5. Physical properties of the formulation

As a result of the pH measurement of the prepared cosmetics, it was confirmed that the pH was 5.32 ± 0.28 . Cosmetics are known to be generally safe without breaking the skin barrier and without irritating the skin when the weak acidity is maintained between pH 5 and pH 6 [98].

Viscosity is the internal frictional force on both sides of the parallel plane and can vary depending on temperature, type and time of liquid, air bubbles, etc. [99]. The result of measuring the viscosity of the prepared cosmetic was confirmed to be 120.020 ± 510.32 . With a general cream formula viscosity, the cream prepared in this study showed a high viscosity stability.

V. Conclusion

In this study, the activity and components of the antioxidants were measured by extracting the fruits and seeds of the Ajwa date palm, which is one of the well-known date palm species found only in the Kingdom of Saudi Arabia, using three methods: hot water, 70% ethanol, and 100% ethanol.

A comparative analysis was performed. It was found that the extract yield was the highest in the extract of fruits and seeds for 100% ethanol extract, with 11.24% for seeds and 5.24% for fruits.

The highest results measured for the antioxidant activity were recorded in the extract of 70% ethanol (DFS2) and 100% ethanol (DFS3) from the seeds and the hot water extract from the fruits (DF1). It was confirmed that the total content of flavonoids and the highest polyphenol content in the fruits and seeds were extracted with hot water (DF1). As a result of HPLC analysis, it was confirmed that the standard polyphenol content was higher in the seed extract than in the fruit extract. However, the gallic acid content was much higher in the hot water fruit extract (DF1). These results are inconsistent with the antioxidant activity and the three extracts from the fruits were weak in the antioxidant tests, unlike the three extracts from the seeds.

This study confirmed that Ajwa date fruit seed extracts have higher antioxidant activity than fruit extracts. Compared with the results of HPLC, which proved the highest content in the hot water extract of the fruits, the antioxidant activity depends mainly on the phenolic compounds in the sample, but it is assumed that there are other unknown compounds and unknown substances involved as well. Therefore, seeds are viewed as a beneficial botanical resource that can be used in the cosmetic and food industries in the future, and it is considered that more research is needed in the future.

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