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EXTRACTION AND ANTIOXIDANT ACTIVITY OF *Carissa carandas* SEED OIL

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ABSTRACT

In this work, Soxhlet and ultrasonic extraction were used to successfully extract oil from *Carissa carandas* seeds cultivated in Ben Tre province, Vietnam. According to the results of the solvent survey, the n-hexane solvent provided high extraction efficiency and high-quality oils. Oil yields were 6.57% after six hours of Soxhlet extraction and 6.69% after one hour of ultrasonic extraction. The study found that adopting ultrasonic extraction technology can assist in minimizing extraction time while maintaining oil quality and production. *Carissa carandas* oil has a refractive index of 1.4568 and a specific gravity of 0.8680 g/cm^3, representing seed oil. The antioxidant activity testing using the DPPH method revealed that the oil concentration of 19.4 mg/mL was capable of inhibiting 50% of DPPH free radicals. Therefore, *Carissa carandas* seed oil has potential applications in food and cosmetics.

Keywords: Carissa carandas, seed oil, Soxhlet extraction, ultrasonic extraction, DPPH antioxidant activity.

1. INTRODUCTION

Carissa carandas (Karonda) is a prominent plant in Vietnam's provinces of Tien Giang, Ben Tre, and Ninh Thuan, as well as other Asian nations such as India, Malaysia, Thailand, Myanmar, and Pakistan. [1] This tree is a creeping, woody, dispersed shrub that grows up to 3-5 m tall, occasionally on top of a big tree, and produces a lot of white, flexible latex [2, 3]. The branches are numerous and extensive, producing dense masses, with sharp thorns up to 5 cm long, in pairs in the leaf axils; the fruit is burgundy until it turns dark purple or virtually black, at which point it is mature. The fruit grows in clusters of three to ten fruits per bunch, and mature fruit is typically available from May to October in Vietnam. Its berry-sized fruits are often used as a condiment or ingredient in Indian pickles and spices. Karonda is a tasty appetizer, and the fruit is pickled before it ripens. Ripe karonda fruit includes a high level of pectin, thus, it is also used to make jelly, jam, squash, syrup, tarts, and chutney, all of which are in high demand in the worldwide market [3]. According to studies, all components of the Karonda plant, including the leaves, fruits, and seeds, contain active chemicals. Because of its high concentration of bioactive compounds such as vitamin C, anthocyanins, flavonoids, glycosides, alkaloids, carbohydrates, sterols, terpenoids, tannins, and saponins, the fruit is used to treat liver dysfunction, reduce fever, anti-diabetic, anti-inflammatory, and heal wounds [2, 3]. However, Karonda seeds have not been noticed and are frequently discarded during the fruit-making process. Each *Carissa carandas* fruit has four to five seeds. Karonda seeds are typically oblong, with a concave endosperm and mushy texture [4]. The particles are 2-3 cm

long and 0.5-1 cm wide. The surface of the seeds is dark brown or black, with a faint shell design. This is a source of high-value waste products that can be used to extract oil from karonda grains used in food or personal care goods to meet the growing demands of humanity.

Figure 1. Carissa carandas fruit and seeds

Vegetable oils are plant-based oils with high fatty acid content. Fatty acids (FAs) play a significant role in the formation and function of the skin's outer layer or epidermis (stratum corneum), which contains glycolipids, intercellular lipids (cement), and a lipid layer known as natural moisturizing factors. Personal care products primarily contain unsaturated fatty acids in triglycerides (TG), particularly EFA linoleic acid (omega-6) and α-linolenic acid (omega-3). Skin and hair are moisturized and softened by utilizing FA-rich vegetable seed oils, which prevent water loss through the epidermis [5]. Between 2000 and 2020, global vegetable oil consumption climbed steadily from around 90.5 million metric tons to 207.5 million metric tons [7]. Coconut, oil palm, soybean, and avocado oil are commercially important plant oil sources [8].

The oil is separated from the plant using technology such as mechanical presses, solvent extraction, supercritical fluid extraction (SFE), extraction aided by ultrasound (UAE), and microwaves (MAE) [9-13]. Mechanical presses produce high-quality oil, but the process often has a low extraction rate and requires a lot of energy [7-8]. Solvent extraction with solvents as *n*-hexane, ethanol, methanol, petroleum ether, and acetone is the major method of extracting oil from vegetables, flowers, and oil seeds [10]. The disadvantages of solvent extraction include the need for more solvents, the high temperature, and the length of the extraction period. To counter this, ultrasonic aided extraction (UAE) is a green and fast oil extraction process that achieves a better extraction rate with less energy. Ultrasound-assisted oil extraction is a popular technology because it is ecologically benign and easy to incorporate with other extraction techniques. In this process, cavitation bubbles form in the solvent and burst, creating pressure and temperature changes that speed up solute mass transfer. The miscella, which includes the solvent and oil mixture, is then desolventized with evaporators before being steam striped to extract the oil.

Each oil is of a different origin and has unique physicochemical qualities that influence its quality, flavor, and application. In Vietnam, vegetable oil research is conducted using seeds from papaya, passion fruit, blind fruit, rapeseed, and other plants. There is currently minimal research on Karonda plants, particularly the seeds. This study assessed the yield of seed oil extracted from Karonda seeds using Soxhlet extraction and ultrasonic extraction. The physicochemical characteristics and antioxidant activity were investigated.

2. MATERIALS AND METHODS

2.1. Materials

The seeds of ripped Karonda fruits were manually removed and rinsed with water before being oven dried at 50 ºC until the moisture level was below 5%. The dried seeds were finely ground and kept in an airtight container, and utilized for further experimentation.

All the chemicals and solvents, such as chloroform (≥99%), hexane (≥95%), acetone, and absolute ethanol utilized for this study. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by Sigma Aldrich.

Figure 2. Extraction systems: (a) Soxhlet extraction and (b) Ultrasonic extraction

2.2. Extraction process

2.2.1. Soxhlet method

Soxhlet oil extraction was conducted for different times, from 30, 60, 90, 150, 180, 240 mins. The extraction process was conducted by weighing exactly 10,0000 ± 0,001g of seed into a thimble tube. The effect of solvents such as chloroform, acetone, ethanol, and *n*-hexane on the oil yield was recorded in the extraction time of 150 minutes. 100 mL of solvents was used during these experiments. After the extraction time, the flask containing solvent and seed oil was transferred to the filtering step.

2.2.2. Ultrasound-Assisted Extraction method

In this method, n-hexane was used for the extraction of karonda seeds. The proportion of seed (sample) to the solvents (R/S) was 1:2, 1:5, 1:10, and 1:15 in 250-mL bottles. The Karonda seed–solvent mixture was treated by ultrasound for 10, 20, 30, 40, 50, 60, and 90 min at the frequency of 35 kHz in the ultrasonic bath (Elmasonic S, Germany) (40% filled with distilled water) at 30 ± 1 °C.

2.2.3. Filtration and concentration

The crude extracts were filtered using a Whatman No. 1 paper filter before being concentrated at 60 °C using a vacuum rotary evaporator (HAHNVAPOR HS-2005V, Korea).

The extracted oils were weighed and stored in the fridge for later examination. Oil yield was calculated as the amount of oil obtained per 100 g of seed weight.

2.3. Determination of physicochemical properties of Karonda seed oil

Physicochemical properties such as specific gravity, refractive index, iodine value, and acid value were calculated by the AOCS official methods [14]. For *antioxidant activity analysis,* a 2,2-diphenyl-1-picrylhdrazyl (DPPH) free radical scavenging activity assay was chosen to measure the antioxidant activity of the oil samples. The extracted oil sample (2 mL) at the different concentrations from 4 mg/mL to 100 mg/mL was mixed with 2 mL of a 0.5 mM DPPH solution and placed in the dark at room temperature for 30 min. The measurements were conducted at 518 nm using a JASCO V-700 UV-VIS spectrometer.

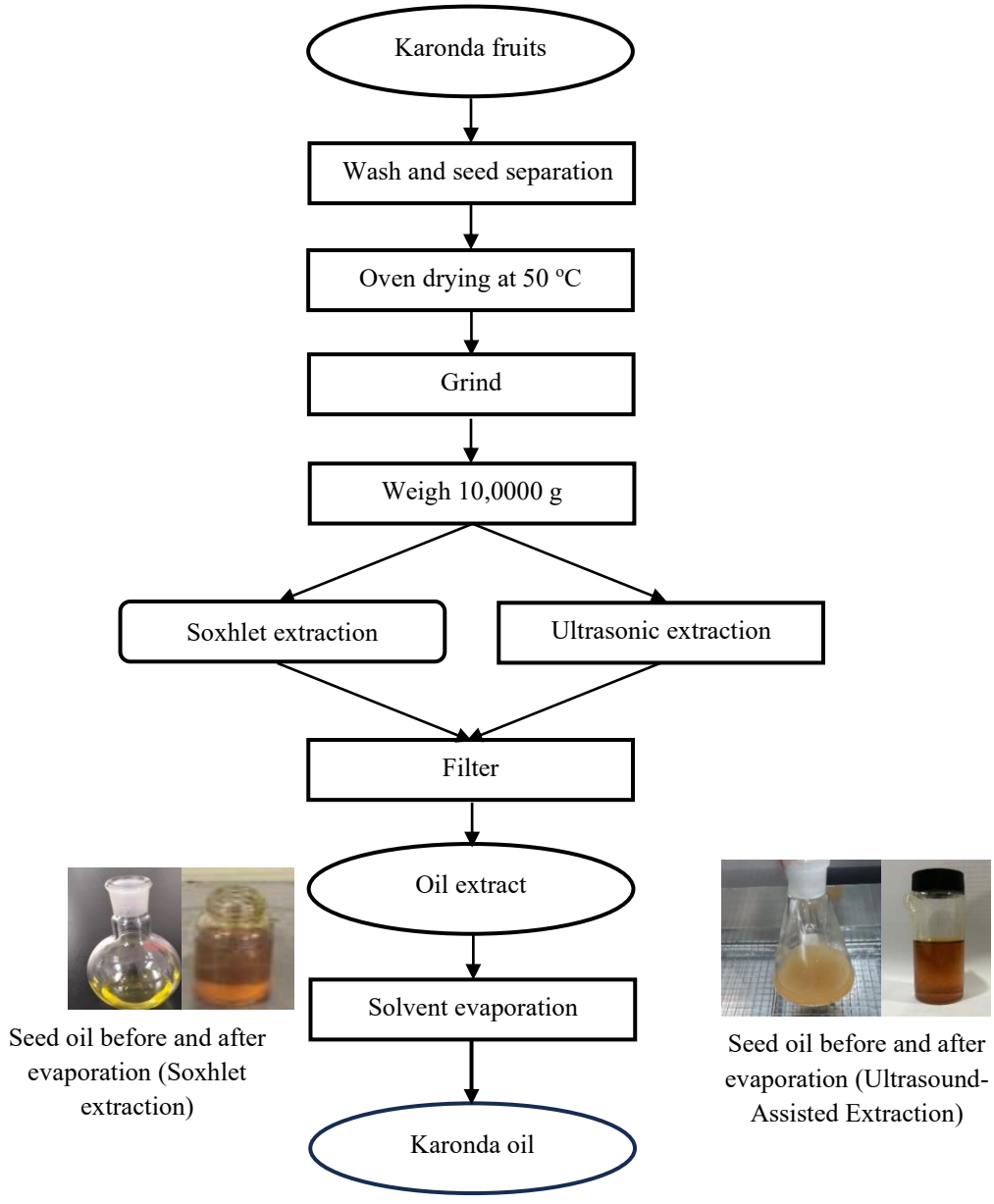

Figure 3. Extraction progress of oil from *Carissa carandas* seeds

3. RESULTS AND DISCUSSION

3.1. Effect of extraction conditions on the oil yield

3.1.1. Effect of solvent

Solvents play a very important role in the extraction process of granular oil because the solvent is able to dissolve oil in the raw material. In this study, Soxhlet extraction (SE) is a conventional method executed for oil extraction from Karonda seed by using different solvents such as hexane, chloroform, ethanol, and acetone. The advantages of these four types of solvents are that they are easy to obtain, inexpensive, and can be completely separated from the oil mixture by the evaporation method. The results of the oil extraction efficiency in Fig. 4 show that the oil yield extracted by hexane and chloroform (4.30 and 4.54%) was higher than by ethanol and acetone (2.95% and 2.22 %). It can be summarized that lipid extraction from Karanda seed by single non-polar solvents was more efficient than by non-polar solvents. This is similar to the study of Christian Cravotto et al. [15]. Chloroform is a probable carcinogen in humans. Chloroform toxicity can lead to central nervous system depression, cardiac arrhythmias, hepatic damage, and hepatic carcinoma [16]. Compared to chloroform, *n*-hexane has been demonstrated to be neurotoxic to humans. It has even been designated as a cause of occupational disorders in numerous European nations since the 1970s [15]. However, hexane is still known to be a promising solvent for oil extraction due to its high-fat solubility, low boiling point (about 68 ◦C), difficulty in dissolving in water, easy recyclability, and relatively low cost [15]. According to EU Directive 2009/32/EC, n-hexane is listed as an extraction solvent that can be used in the production of foods or food products [17]. In this study, the oil efficiency extraction between *n*-hexane and chloroform was different insignificantly (p-value > 0.05). Therefore, *n*-hexane was selected for the next experiments.

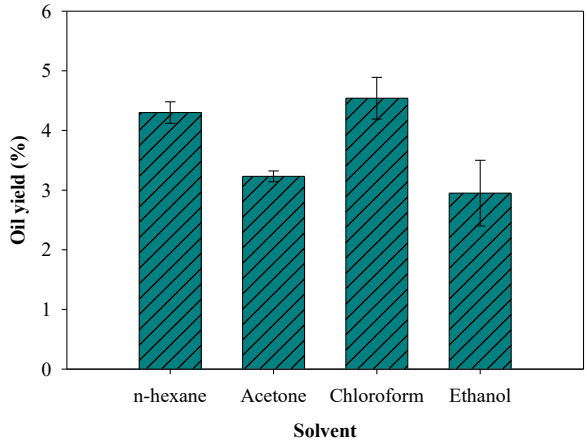

Figure 4. Effect of different solvents on oil extraction

3.1.2. Effect of extraction time on oil content by Soxhlet extraction method and Ultrasonic extraction method

Optimization of extraction time for any unit operation is very important as it affects the production rate and process economy. In this experiment, the effect of the Soxhlet extraction method and the ultrasonic extraction method on oil efficiency and extraction time was evaluated. Soxhlet extraction maintains solvent extraction efficiency and high efficiency by continually extracting solid materials with pure solvent using the solvent reflux and siphon

mechanism [18]. After placing the solid sample on a filter paper in the shape of a thimble, the Soxhlet extractor is assembled. The solvent reservoir flask is added with solvent and placed on a heating mantle. Following heating, the sample powder and the solvent's condensed vapors come into contact, combining the powder's soluble portion with the solvent for extraction. The solvent holding the extract is siphoned back when the solvent surface rises above the siphon's maximum height. The extraction procedure is done several times till the extraction time is complete. For ultrasonic extraction (also called ultrasound-assisted extraction, UAE), under the influence of high-frequency sound waves (typically 20–100 kHz), break cell walls or membranes and increase solvent penetration into cells. This helps to release oil into the solvent. Therefore, the mass transfer of compounds into the solvent was improved, and the extraction time became shorter.

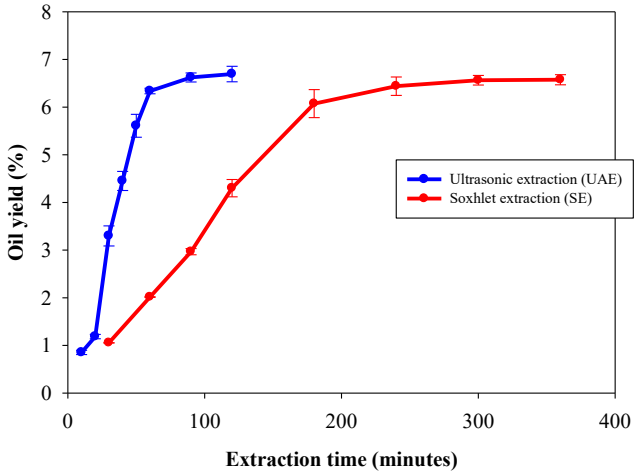

Figure 5. Results of oil content survey by extraction time: (a) Sohxlet extraction method, (b) Ultrasonic extraction method.

The results of the effect of two extractions on the Karonda oil yield are shown in Fig. 5. The process time from 30 min to 360 min was studied for the extraction of oil using a Soxhlet apparatus. The oil extraction efficiency of the SE method increased from 1.05% to 6.43% as the extraction time gradually increased from 30 min to 240 min. After 240 minutes, the oil extraction efficiency reached saturation with a negligible increase from 6.44% to 6.57% seed oil yield.

As a comparison, the extraction technique with the support of ultrasonic waves (UAE) is performed. Oil extraction efficiency of the UAE method increases from 0.85% to 6.34% as the extraction time gradually increases from 10 minutes to 60 minutes. In the range from 60 minutes to 120 minutes, the oil extraction efficiency increased slowly from 6.34% to 6.69%. After 30 min of extraction, UAE gave the oil yield of 3.30 %, which was higher than three times that of SE (1.05%). Similarly, after 60 minutes, the oil efficiency of UAE and SE were 6.34 and 2.02, respectively. The study shows that the ultrasonic extraction technique also has a shorter extraction time and higher efficiency than the Soxhlet extraction method. The quality of the oil extracted by the two extraction methods is light brown. Therefore, the ultrasonic extraction method greatly reduces the cost and time in the process of extracting vegetable oils. As a consequence, the ultrasonic extraction and extraction time of 60 minutes were chosen for the oil extraction from the Karonda seed in the next experiments.

3.1.3. Effect of raw material to solvent ratio in UAE extraction

n-hexane was added to the dry powder as part of the ultrasonic wave modification, and the cells absorbed the solvent. A lower solvent concentration will make the solution more viscous, and poor mass transfer that results from increased viscosity will reduce the extraction yield. With a high solvent-to-sample ratio, mass transfer rises. High concentration gradient, high osmotic pressure, and increased driving power could be the cause. Therefore, the extraction efficiency increases. Similar results were also reported by Tugba et al. [18] and Milan D. Kostic [9]. The use of more solvents raises the process cost and pollutes the environment due to their toxicity. Therefore, optimization of the R/S ratio is an important parameter, and it is expressed in Figure 6.

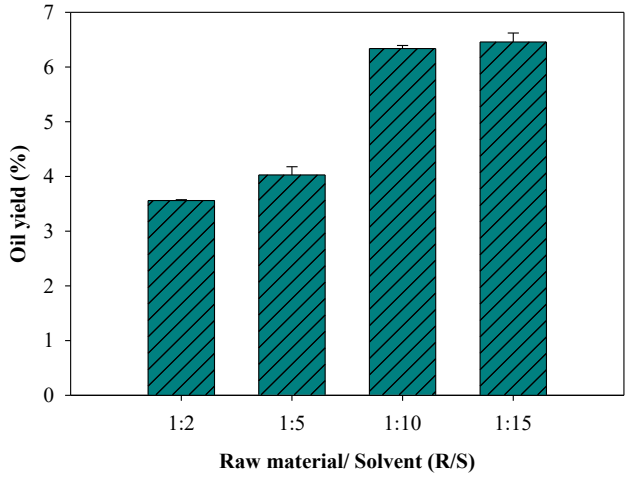

Figure 6. Effect of raw material /solvent on oil yield by UAE

Figure 6 demonstrated that the best ratio was 1:10 (w/v), resulting in a yield of 6.37% oil extraction after 60 min of extraction. The yield has increased by around 50% when the R/S ratio was changed from 1:2 to 1:10. A negligible change in extraction yield is provided by the ratio shift from 1:10 to 1:15. This is because the extraction process has reached a balance, the fatty oil has been completely extracted from the grain when at a material/solvent ratio of 1:10. The increase in solvent volume only causes waste and wastes her time chasing the solvent. A material/solvent ratio of 1:10 was selected for further studies.

In summary, the maximum oil yield in Karonda seed oil was 6.69%, corresponding to an oil recovery of 100% in 2 hours by ultrasound-assisted extraction and 98% in 6 hours by Soxhlet extraction. The oil content of *Carissa carandas* seed is lower than the previous study of Jayashree Gade et al., with 37.19% by Soxhlet extraction in 6 hours [19], and the study with the oil yield in Soxhlet extraction by petroleum ether of 14.4% [20]. This is explained by the origin, growth conditions, geographical factors, and seed thickness. For comparison, the maximum oil yield of European cranberry bush of 7.44 % was found at 30 min extraction time, 60 W power, and 5 (g/100 mL) solid/solvent ratio [17]. In the study of Ivana Dimić et al., the oil extracted from cherry seeds by Supercritical Fluid Extraction was reported to be 2.50 to 13.02% of oil, depending on the chosen extraction technique and process parameters [21]. Although such seeds are not preferred for large-scale oil production due to their low yield, they are often valued for the unique properties of their oils, which are rich in antioxidants, polyunsaturated fatty acids, or bioactive compounds. In this context, Karonda seed oil, despite its low yield, may hold promise in specialty health products or cosmetic applications, where quality and functionality are prioritized over volume.

3.2. Physical and chemical properties of Karonda seed oil

The quality parameters of the Karonda oil in Table 1 show that the product exhibits a transparent light yellowish-brown color, indicating a high level of purity and minimal impurities. The measured density of 0.8680 g/cm³ falls within the typical range for vegetable oils. The refractive index of 1.4568 also matches the usual values for natural vegetable oils, reflecting a lipid composition predominantly consisting of triglycerides [19]. However, the acid value is relatively high at 38.39 mg KOH/g, suggesting significant free fatty acid (FFA) content, indicating considerable hydrolytic and lipolytic activity [22]. The iodine value of 37.08 mg I_2/100g indicates that the oil is rich in saturated fatty acids, conferring good oxidative stability but lower nutritional value in terms of unsaturated fatty acids. The saponification value of 188.2 mg KOH/g aligns with typical ranges for oils containing medium- to long-chain fatty acids [23]. Moreover, the ester value (149.81 mg KOH/g) confirms that most of the lipid fraction exists in esterified forms such as triglycerides. Overall, the chemical profile of vegetable oil suggests suitability for cosmetic, pharmaceutical, or industrial applications; however, further refining would be necessary to reduce the FFA content if the oil is intended for edible use.

Table 1. Some quality indicators of seed oil

Parameter	Unit	Result
Color		Transparent, light yellowish brown
Density	(g/cm^3)	0.8680
Refractive index (RI)		1.4568 ± 0.0006
Acid value	mg KOH/g	38.4 ± 2.8
Iodine value	mg I_2/100g	37.1 ± 1.9
Saponification value	mg KOH/g	188.2 ± 10.7
Ester value	mg KOH/g	151.1 ± 8.8

3.3. The antioxidant activity of seed oil

Figure 7 depicts the DPPH radical scavenging activity of Karonda oil at dilutions ranging from 4 to 80 mg/mL. According to the survey results, Karonda oil's antioxidant activity can reach 87% when the oil content is 41 mg/mL or higher. The standard curve for Karonda oil concentration ranges from 4 mg/mL to 40.7 mg/mL using the equation $Y = 1.7435X + 16.125$, with a high correlation coefficient $R^2 > 0.99$. The standard curve results indicated that the extract can inhibit up to 50% of DPPH free radicals at a diluted concentration of 19.4 mg/mL. To evaluate the process control, the vitamin C control substance was tested for anti-DPPH activity. Previous research on the chemical composition of Karonda seed oil found that it included 9Z, 12Z-Octadecadienoic acid (Linoleic acid) and 9Z-Octadecenoic acid (oleic acid), as well as unsaturated fatty acids [24] and showed excellent antioxidant activity [25]. This seed oil is appropriate for cosmetic applications.

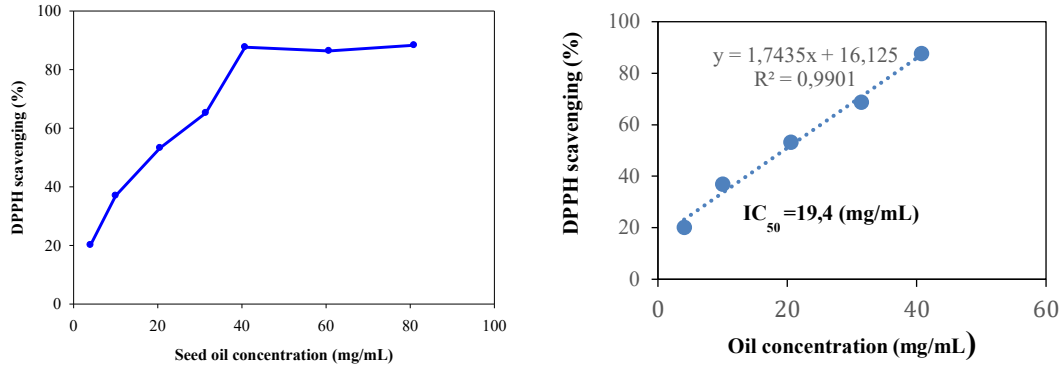

Figure 7. DPPH antioxidant activity of Karonda oil at different concentrations

Figure 8. Oil samples after reacting with DPPH solution for 30 mins

4. CONCLUSION

In this study, plant oil was successfully extracted from the seeds of *Carissa carandas* fruits after production. The oil extraction method is a key factor in determining oil yield and time. The solvent effect revealed that n-hexane produced good extraction efficiency and high-quality oils. Oil yields were 6.57% after six hours of Soxhlet extraction and 6.69% after two hours of ultrasonic extraction using *n*-hexane as the solvent. The ultrasonic extraction technology significantly reduces the cost and time spent extracting vegetable oils. Karonda seeds, a waste material, were found to be an excellent source of oil. Besides, a DPPH antioxidant activity of Karonda seed oil with an IC_{50} of 19.4 mg/mL implied the application of this seed oil in cosmetics, although of low oil extraction.

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TÓM TẮT

NGHIÊN CỨU TRÍCH LY VÀ ĐÁNH GIÁ HOẠT TÍNH KHÁNG OXY HÓA CỦA DẦU HẠT SI RÔ *(CARISSA CARANDAS)*

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Trong nghiên cứu này, phương pháp chiết Soxhlet và chiết siêu âm đã được sử dụng để trích ly thành công dầu từ hạt si rô (*Carissa carandas*) được trồng tại tỉnh Bến Tre, Việt Nam. Theo kết quả khảo sát dung môi chiết, dung môi n-hexan cho hiệu suất trích ly và chất lượng dầu cao. Hiệu suất dầu chiết được là 6,57% sau sáu giờ trích ly bằng phương pháp chiết Soxhlet và 6,69% sau một giờ chiết bằng phương pháp siêu âm. Nghiên cứu cho thấy việc áp dụng công nghệ chiết siêu âm hỗ trợ giảm thời gian trích ly trong khi vẫn duy trì chất lượng và sản lượng dầu. Dầu có chỉ số khúc xạ là 1,4568 và khối lượng riêng là 0,8680 g/cm^3, đặc trưng cho tính chất của dầu hạt. Thử nghiệm hoạt tính chống oxy hóa bằng phương pháp DPPH cho thấy nồng độ dầu là 19,4 mg/mL có khả năng ức chế 50% gốc tự do DPPH. Do đó, dầu hạt si rô (*Carissa carandas*) có tiềm năng ứng dụng trong thực phẩm và mỹ phẩm.

Từ khóa: Carissa carandas, dầu hạt, chiết Soxhlet, chiết siêu âm, hoạt tính kháng oxy hóa DPPH.