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Research paper

# Exploring the Bioactive and Thermal Properties of Buckthorn Seed Oil: A Comprehensive Analysis

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**ABSTRACT**

This study provides a detailed analysis of Buckthorn seed oil, examining its chemical composition, antioxidant properties, antimicrobial activity, and thermal stability before and after heating. GC/MS analysis identified significant components, including Tetracosane, 11-decyl- and Octadecane, 3-ethyl-5-(2-ethylbutyl)-, while new compounds such as Nonacosane and Pentacosane, 13-undecyl- emerged post-heating. The oil exhibited moderate antioxidant activity, with a radical scavenging activity (RSA) of 62%, which was lower than the synthetic standard, Propyl Gallate. Antimicrobial testing showed limited efficacy, with slight sensitivity against *Staphylococcus aureus*, and no observed activity against *Escherichia coli* or *Candida albicans*. DSC analysis indicated a melting point of 40°C and thermal stability up to 150°C, supporting its potential for industrial applications in cosmetics and food processing. Despite its favorable thermal stability and chemical profile, the oil's relatively low bioactivity suggests the need for further enhancement to increase its potential utility in broader applications.

## 1. Introduction

Buckthorn (Rhamnus), a shrub found in Europe and parts of Asia, has long been valued in traditional medicine for its medicinal properties, with its bark and berries commonly used as a laxative and diuretic (Nigussie et al., 2021). More recently, the oil extracted from buckthorn seeds has attracted attention due to its rich chemical makeup, particularly its abundance of essential fatty acids, phytosterols, and tocopherols (Yue et al., 2017). These bioactive compounds make buckthorn seed oil a promising ingredient in health and cosmetic products, offering antioxidant, anti-

inflammatory, and antimicrobial benefits. The rising demand for plant-based, sustainable, and multifunctional products in the cosmetic and nutraceutical sectors has encouraged further research into plant oils like buckthorn (Nekkaa et al., 2021, Guzmán et al., 2021).

Buckthorn seed oil is notably rich in linoleic acid (omega-6) and oleic acid (omega-9), both of which are essential for maintaining skin health, reducing inflammation, and supporting cellular regeneration (Yang et al., 2001). It also contains significant amounts of phytosterols like beta-sitosterol, which is known for lowering cholesterol levels and providing cardioprotective effects (Li et al., 2007). These attributes make the oil especially beneficial in skincare formulations aimed at improving skin health and combating oxidative stress (Lin et al., 2017). Additionally, the high levels of tocopherols (vitamin E) present in buckthorn seed oil enhance its antioxidant properties, helping to neutralize free radicals that contribute to premature skin aging and environmental damage (Mazhar et al., 2013).

From a thermal perspective, understanding how buckthorn seed oil responds to heat is crucial, particularly for its applications in industries such as food and cosmetics, where thermal processing is involved (Cai et al., 2021). Exposure to heat can lead to the oxidation of fatty acids and the breakdown of sensitive compounds like tocopherols, which in turn reduces the oil's effectiveness and shelf life (Silva et al., 2010). Gas Chromatography-Mass Spectrometry (GC/MS) is often used to analyze the chemical composition of the oil before and after heating, providing insights into the stability of its bioactive components (Pastor et al., 2020). Research has shown that heating oil's rich in polyunsaturated fatty acids, like buckthorn seed oil, can significantly alter their chemical structure, which may reduce their bioactivity (Kostik et al., 2013).

The thermal properties of buckthorn seed oil are vital to its use in the food and cosmetic industries, where it is often subjected to high temperatures during processing or storage (Warner 1999). Differential Scanning Calorimetry (DSC) is a key technique for evaluating the thermal stability of oils, as it measures phase transitions such as melting and crystallization, which can affect the texture and stability of products that incorporate the oil (Pardauil et al., 2011). Understanding the oil's melting and crystallization points can help optimize formulations that undergo heat processing, such as emulsions and creams (Pavlačková et al., 2018).

Beyond its chemical composition and thermal properties, buckthorn seed oil has demonstrated significant antimicrobial activity, making it valuable for developing natural preservatives in skincare and food products (Murbach Teles Andrade et al., 2014). Studies have shown that plant oils high in unsaturated fatty acids, such as buckthorn seed oil, can inhibit the growth of harmful bacteria, including Staphylococcus aureus and Escherichia coli. However, the effects of heating on the antimicrobial properties of buckthorn seed oil remain underexplored, and this study seeks to fill that gap by examining the oil's efficacy before and after thermal exposure (Gliszczynska-Swiglo et al., 2007).

In addition, buckthorn seed oil's antioxidant activity enhances its value, as antioxidants are essential in preventing oxidative damage, which can lead to chronic diseases and premature aging (Suanarunsawat et al., 2009). The high tocopherol content in the oil is primarily responsible for its ability to neutralize free radicals, but other components, such as phytosterols, may also contribute to its antioxidant capacity. This research will assess the oil's antioxidant activity before and after heating to determine how thermal exposure affects its bioactive properties.

The objectives of this research are to evaluate the chemical composition of buckthorn seed oil before and after heating using GC/MS, identifying any changes in key components like fatty acids, tocopherols, and phytosterols, to assess its thermal stability using DSC to understand its behavior at various temperatures, and to investigate the oil's antimicrobial and antioxidant properties, focusing on its ability to inhibit microbial growth and protect against oxidative damage. The results of this study will contribute to a better understanding of buckthorn seed oil's potential uses in cosmetics, nutraceuticals, and other industries where heat and bioactivity are critical considerations.

## 2. Materials and Methods

### *2.1 Plant material*

Buckthorn seeds were acquired at a local market in Khartoum city, Sudan. And it was identified at the Department of Phytochemistry and Taxonomy (National Research Centre, Khartoum-Sudan).

### *2.2 Extraction of oil's (Sample Preparation)*

The buckthorn seeds were first washed and dried to reduce their moisture content. After drying, 600 grams of the seeds were finely ground to increase the surface area for oil extraction. The powdered seeds were then immersed in n-hexane at a 1:3 (w/v) ratio for 24 hours, with continuous stirring to facilitate the extraction process. Once steeping was complete, the mixture was filtered to separate the solvent-oil extract from the seed residues. The solvent was removed using a rotary evaporator at a reduced pressure and a temperature between 40-50°C, minimizing any potential oil degradation. The resulting oil (50 ml) was collected, weighed, and

stored in amber-colored bottles at 4°C until further analysis (Ixtaina et al., 2011).

### *2.3 GC/MS Analysis*

The chemical composition of the seed oil was assessed using the Agilent 7890B-5977A GC/MS system. The HP-5ms column (30 m x 250 µm x 0.25 µm) was employed, capable of temperatures from 0°C to 325°C (maximum 350°C). Helium served as the carrier gas at 1 mL/min flow rate. The temperature program started at 50°C for 3 minutes, then ramped up by 15°C/min to 150°C, followed by 5°C/min to 180°C, and 8°C/min to 325°C, where it was held for 10 minutes. The injection port was set at 280°C, using 1 µL spitless injections. The mass spectrometry analysis was performed in electron ionization mode (70 eV) at 230°C, with a solvent delay of 3.5 minutes. Data acquisition spanned an m/z range of 35-600, with the quadrupole temperature set at 150°C. The NIST 17 database was used for compound identification. The oil samples were also heated at 120°C for one hour, and the same analysis process was repeated (Assaggaf et al., 2023).

### *2.4 DSC Analysis*

The thermal properties of buckthorn seed oil were examined using Differential Scanning Calorimetry (DSC). A sample of approximately 5-10 mg of the oil was placed in aluminum pans, and subjected to a controlled heating cycle, from -50°C to 200°C at different heating rates (10°C/min, 20°C/min, and 40°C/min). Once reaching 200°C, the sample was cooled back to -50°C and then reheated to 200°C to confirm repeatability and detect any additional thermal events. The DSC thermograms captured thermal transitions, including melting and crystallization temperatures, as well as enthalpy changes, providing insight into the oil's thermal stability and potential applications. The thermograms were compared to analyze the effects of varied heating rates on the oil's properties (Tan et al., 2000).

### *2.5 Antimicrobial assay*

The antimicrobial activity of the essential oil was evaluated against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* using the disc diffusion method. Sterile filter paper discs (6 mm in diameter) were prepared with 10 µL and 50 µL of essential oil, respectively. Negative controls with 10 µL of saline and positive controls with Levofloxacin-sensitive tablets (5 µg) were included. Based on the "Antimicrobial Susceptibility Test Implementation Standard 2010," inhibition zones were classified as: >20 mm (severe sensitivity), 15-20 mm (high sensitivity), 10-14 mm (medium sensitivity), <10 mm (low sensitivity), and 0 mm (resistance). Bacterial and fungal suspensions were grown in nutrient broth until OD600 reached 0.5 (approximately 10^8 CFU/mL). After spreading 100 µL of the suspension onto nutrient agar plates, the discs were placed, incubated, and the inhibition zones were measured in millimeters (Balouiri et al., 2016).

### *2.6 DPPH Radical Scavenging Assay*

The antioxidant activity of the samples was measured using the DPPH radical scavenging assay, based on the method described by Brand-Williams et al. (1995) with minor modifications. In a 96-well plate, the test materials were reacted with 2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH), a stable free radical, for 30 minutes at 37ºC. The concentration of DPPH was set at 300 µM. The test samples were dissolved in DMSO, and DPPH was prepared in ethanol. After incubation, the absorbance was measured at 517 nm using a spectrophotometer, and the percentage of radical scavenging activity was calculated relative to a DMSO-treated control group.

## 3. Results

### *3.1 Chemical Composition Analysis for buckthorn oil*

#### 3.1.1 Before heating

The chemical composition of Buckthorn seed oil was analyzed using GC/MS both before and after heating. Before heating, the major constituents were Tetracosane, 11-decyl- (32.78%), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (30.54%), and Pentacosane (7.53%), alongside smaller quantities of Docosane, 11-butyl- (5.37%) and Isopropyl myristate (4.46%). Unidentified compounds constituted 15.35% of the fraction. the mass spectra of the four major compounds are provided in Figures (3, 4, 5, and 6).

#### 3.1.2 After heating

After heating, the composition shifted, with Octadecane, 3-ethyl-5-(2-ethylbutyl)- increasing to 35.62%, while Tetracosane, 11-decyl- dropped significantly to 4.19%. New compounds like Nonacosane (12.49%) and Pentacosane, 13-undecyl- (9.76%) appeared. Additionally, three unidentified compounds emerged at retention times 26.77, 27.18, and 27.61 minutes, with concentrations of 4.18%, 6.19%, and 4.98%, respectively. These changes indicate the thermal transformation of the oil's components.

#### 3.1.3 The impact of heating on Buckthorn seed oil

reveals significant alterations in its chemical composition, likely due to the thermal degradation of sensitive compounds and the formation of new molecules. The increase in Octadecane, 3-ethyl-5-(2-ethylbutyl)-

after heating suggests that this compound exhibits strong thermal stability, maintaining or increasing in concentration under heat. In contrast, Tetracosane, 11-decyl- was significantly reduced, indicating its susceptibility to thermal breakdown. The appearance of new compounds, such as Nonacosane and Pentacosane, 13-undecyl-, likely results from the decomposition or rearrangement of other hydrocarbons. Moreover, the emergence of three unidentified compounds at significant concentrations suggests potential thermal transformations that create new chemical structures not present in the unheated oil. These results underscore how heat processing can alter the chemical profile of the oil, potentially impacting its functional properties in applications such as cosmetics or pharmaceuticals.

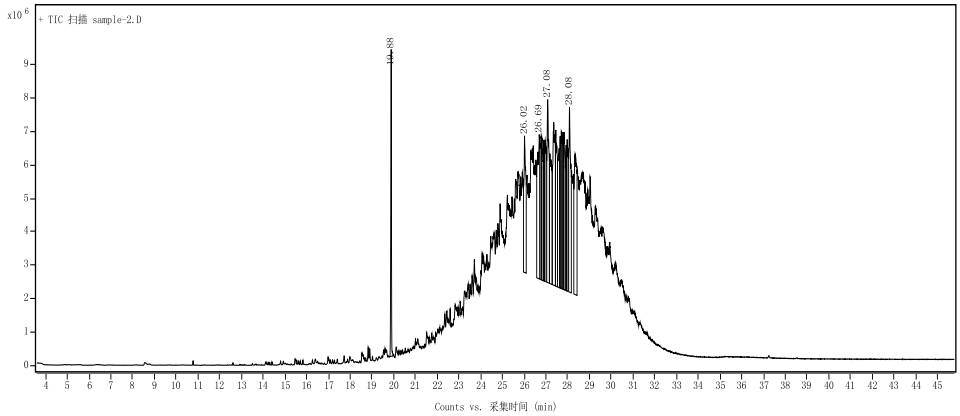


**Fig. 1** GC/MS chromatogram of Buckhorn oil before heating

**Table 1** Major chemical constituents of Buckhorn oil before heating

| R. time | Compound | M.F | M.W g/mol | % | Area% |
|---|---|---|---|---|---|
| 19.88 | Isopropyl myristate | C17H34O2 | 270.45 | 4.46 | 54.4 |
| 26.02 | Docosane, 11-butyl- | C26H54 | 366.71 | 5.37 | 65.52 |
| 26.69 | Tetracosane, 11-decyl- | C34H70 | 478.92 | 7.2 | 87.9 |
| 26.77 | Un known Compound | | | 4.18 | 51.04 |
| 26.82 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 3.95 | 48.27 |
| 26.91 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 2.59 | 31.62 |
| 26.96 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 4.07 | 49.7 |
| 27.08 | Tetracosane, 11-decyl- | C34H70 | 478.92 | 7.14 | 87.15 |
| 27.18 | Un known Compound | | | 6.19 | 75.59 |
| 27.36 | Tetracosane, 11-decyl- | C34H70 | 478.92 | 8.19 | 100 |
| 27.45 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 5.36 | 65.48 |
| 27.61 | Un known Compound | | | 4.98 | 60.83 |
| 27.65 | Tetracosane, 11-decyl- | C34H70 | 478.92 | 3.27 | 39.86 |
| 27.7 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 3.41 | 41.57 |
| 27.77 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 3.47 | 42.41 |
| 27.83 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 4.66 | 56.91 |
| 27.93 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 3.03 | 37.04 |
| 27.97 | 8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3.beta.-methoxy-4,4-dimethyl- | C24H36O6 | 418.53 | 3.95 | 48.16 |
| 28.08 | Tetracosane, 11-decyl- | C34H70 | 478.92 | 6.98 | 85.17 |
| 28.34 | Pentacosane | C25H52 | 352.69 | 7.53 | 91.9 |

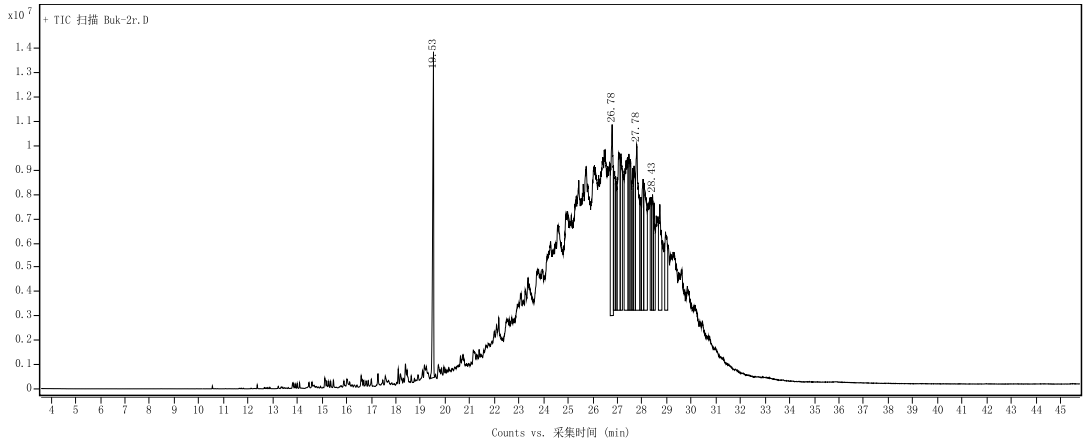


**Fig. 2** GC/MS chromatogram of components in Buckhorn oil after heating

**Table 2** Major chemical constituents of Buckhorn oil after heating

| R. time | Compound | M.F | M.W g/mol | % | Area % |
|---|---|---|---|---|---|
| 19.53 | Isopropyl myristate | C17H34O2 | 270.45 | 6.08 | 62.26 |
| 26.78 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 8.24 | 84.39 |
| 26.84 | Un known Compound | | | 4.16 | 42.6 |
| 26.93 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 3.56 | 36.46 |
| 27.07 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 7.35 | 75.29 |
| 27.14 | Docosane, 7-hexyl- | C28H58 | 394.77 | 5.26 | 53.92 |
| 27.43 | Nonacosane | C29H60 | 408.81 | 9.06 | 92.84 |
| 27.46 | Un known Compound | | - | 4.59 | 46.99 |
| 27.52 | Tetracosane, 11-decyl- | C34H70 | 478.93 | 4.19 | 42.93 |
| 27.63 | 17-Pentatriacontene | C35H70 | 490.94 | 3.02 | 30.97 |
| 27.68 | Heptadecane, 9-hexyl- | C23H48 | 324.64 | 4.47 | 45.78 |
| 27.78 | Pentacosane, 13-undecyl- | C36H74 | 506.99 | 9.76 | 100 |
| 27.95 | Un known Compound | | 394.77 | 2.76 | 28.25 |
| 28.04 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 4.27 | 43.8 |
| 28.09 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 6.62 | 67.87 |
| 28.36 | Un known Compound | | | 2.77 | 28.38 |
| 28.43 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 2.98 | 30.49 |
| 28.49 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | C26H54 | 366.71 | 2.6 | 26.69 |
| 28.72 | 2-Methylheptacosane | C28H58 | 394.77 | 4.84 | 49.57 |
| 28.95 | Nonacosane | C29H60 | 408.81 | 3.43 | 35.12 |

**Table 3** Template for comparing the GC/MS results of buckthorn seed oils (2) before and after heating

| Component Name | Before Heating | After Heating | % Change | Notes |
|---|---|---|---|---|
| Isopropyl myristate | Present | Present | Increased from 4.46% to 6.08% | Increase suggests thermal stability |
| Octadecane, 3-ethyl-5-(2-ethylbutyl) | Present | Present | Increased from 30.54% to35.62% | Consistent presence with minimal increase |
| Tetracosane, 11-decyl- | Present | Present | Decreased from 32.78% to 4.19% | Significant drop; potential decomposition |
| Docosane, 11-butyl- | Present | Absent | Not detected | Completely damaged or transformed |
| 8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3.beta.-methoxy-4,4-dimethyl | Present | Absent | Not detected | Likely deteriorated at higher temperatures |
| Pentacosane | Present | Absent | Not detected | Complete loss, presumably thermal decomposition |
| Nonacosane | Not present | Present | Newly detected | Formation due to thermal reactions |
| 17-Pentatriacontane | Not present | Present | Newly detected | Newly generated compound after heating |
| Heptadecane, 9-hexyl- | Not present | Present | Newly detected | Formation suggests heat-induced synthesis |
| Pentacosane, 13-undecyl- | Not present | Present | Newly detected | Likely produced as a result of compound alterations |
| 2-Methylheptacosane | Not present | Present | Newly detected | Presence indicates complicated thermal reactions |

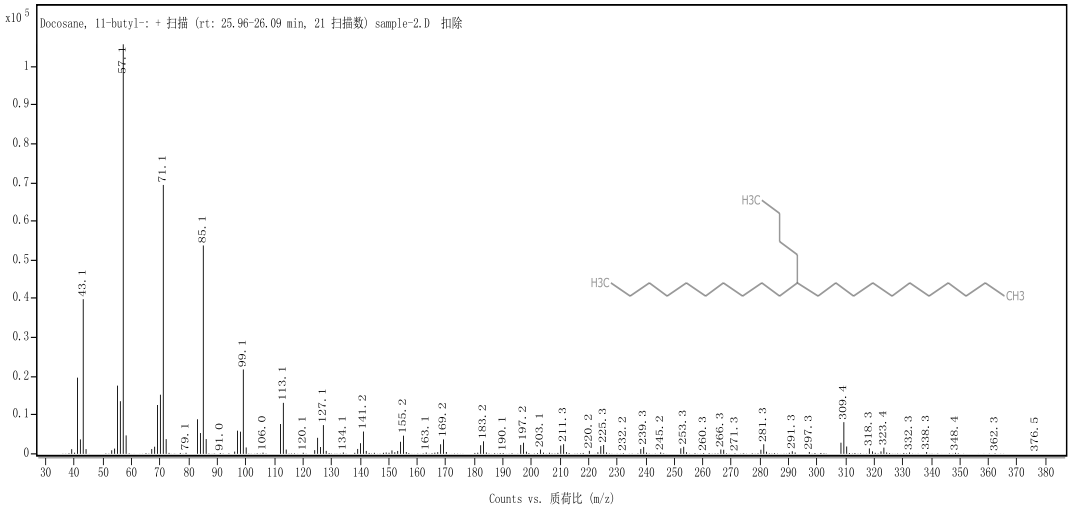


**Fig. 3** Mass spectrum of Docosane, 11-butyl-

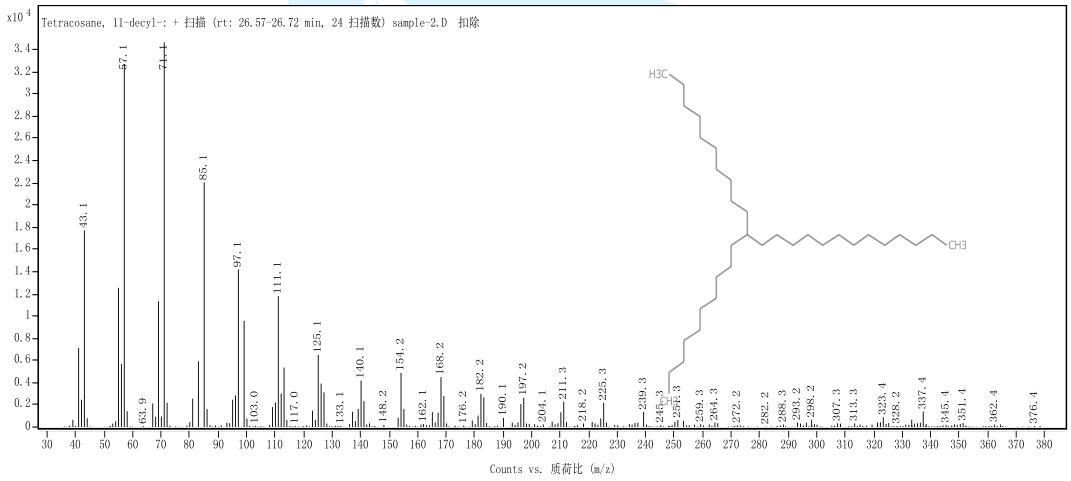


**Fig. 4** Mass spectrum of Tetracosane, 11-decyl-

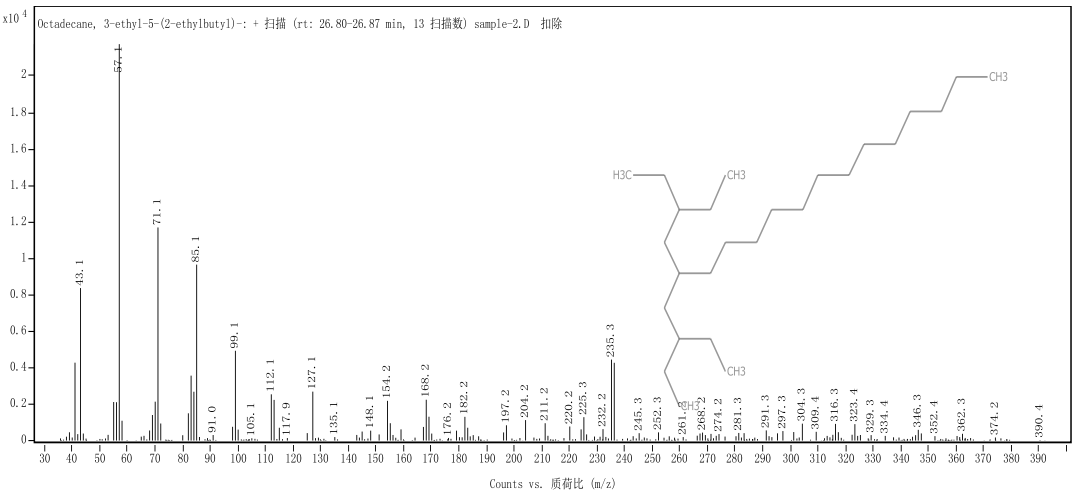


**Fig. 5** Mass spectrum of Octadecane, 3-ethyl-5-(2-ethylbutyl)-

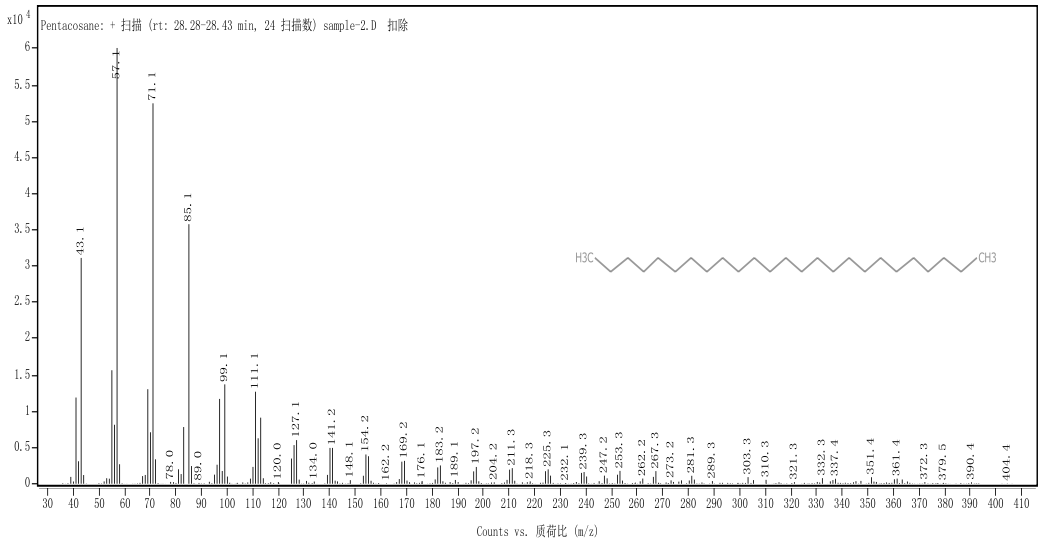


**Fig. 6** Mass spectrum of Pentacosane

### *3.2 Antimicrobial Activity*

The antimicrobial testing of buckthorn seed oil demonstrated limited effectiveness against the microorganisms studied. At a 10 µL concentration, the oil showed mild activity against *Staphylococcus aureus*, with a 9.3 mm inhibition zone. However, when the concentration was increased to 50 µL, the bacteria exhibited resistance, suggesting that the bioactive components in the oil might not be potent enough to maintain inhibitory effects at higher concentrations. For *Escherichia coli* and *Candida albicans*, the oil showed resistance at both tested volumes, indicating minimal antibacterial and antifungal properties. This lack of significant antimicrobial activity could be attributed to either the low concentration of bioactive compounds, such as tocopherols and sterols, or their degradation during processing. While buckthorn oil shows some inhibitory potential against *S. aureus*, further investigation is needed to enhance its efficacy, possibly through combining it with other active agents or improving the extraction process.

**Table 4** Antimicrobial Activity of Buckthorn Seed Oil

| Microorganism | Volume of Oil (µL) | Inhibition Zone (mm) | Sensitivity |
|---|---|---|---|
| *Staphylococcus aureus* | 10 | 9.3 mm | Low Sensitivity |
| *Staphylococcus aureus* | 50 | - | Resistant |
| *Escherichia coli* | 10 | - | Resistant |
| *Escherichia coli* | 50 | - | Resistant |
| *Candida albicans* | 10 | - | Resistant |
| *Candida albicans* | 50 | - | Resistant |

### *3.3 Antioxidant activity*

The antioxidant activity of Buckthorn Oil was assessed using the DPPH method with Propyl Gallate as the control, the table showing the %RSA (Radical Scavenging Activity) ± SD (Standard Deviation) values for the antioxidant activity.

**Table 5** The antioxidant activity of Buckthorn seeds oil

| Sample | %RSA±SD (DPPH) µg/ml |
|---|---|
| Buckthorn Oil | 62 % ± 1.9 µg/mL |
| Propyl Gallate (Standard) | 90.5% ± 0.8 µg/mL |

The antioxidant activity of Buckthorn seed oil was measured using the DPPH radical scavenging assay, with results expressed as a percentage of radical scavenging activity (%RSA). Buckthorn seed oil exhibited a moderate antioxidant activity of 62% ± 1.9 µg/mL, which, although significant, was lower compared to the synthetic standard Propyl Gallate, which showed a higher %RSA of 90.5% ± 0.8 µg/mL. These results indicate that while buckthorn oil has notable antioxidant potential, it is less effective than Propyl Gallate under the same experimental conditions. This moderate antioxidant capacity may still be beneficial in various applications, particularly where natural antioxidants are preferred over synthetic alternatives.

### *3.4 Explanation of DSC Results*

The Differential Scanning Calorimetry (DSC) analysis of buckthorn seed oil conducted at heating rates of 10K/min, 20K/min, and 40K/min revealed significant thermal behavior. Initially, an endothermic peak around 40°C represents the melting point of specific oil components. The oil exhibited thermal stability between 70°C and 150°C, where no notable transitions were detected, indicating its resilience within this range. Above 150°C, a rise in heat flow occurred, corresponding to continued melting or degradation of oil components. The varying heating rates demonstrated that higher rates, such as 40K/min, led to more intense thermal reactions and increased heat flow compared to lower rates. These findings underscore that buckthorn oil melts around

40°C, remains stable up to 150°C, and undergoes further thermal changes at higher temperatures, making it ideal for use in food processing, cosmetics, and pharmaceuticals.

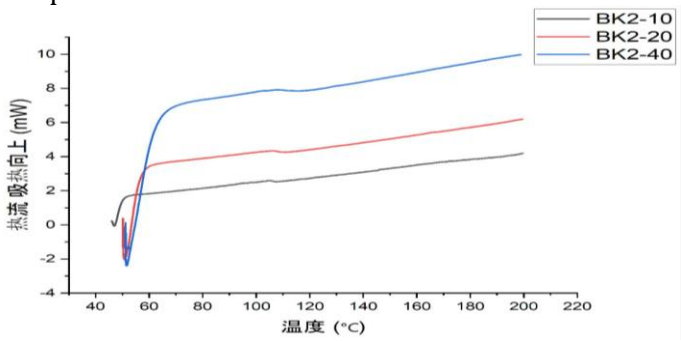


**Fig. 7** DSC Thermograms for Buckhorn oil

## 4. Discussion

Upon comparing the chemical composition of Buckthorn seed oil before and after heating, it's clear that heating causes significant alterations. Prior to heating, the oil was primarily composed of Tetracosane, 11-decyl- and Octadecane, 3-ethyl-5-(2-ethylbutyl)-, but heating led to the formation of new compounds, such as Nonacosane and Pentacosane, 13-undecyl-, indicating chemical transformations. Similar results were observed by Salimon et al. (2012) and Tan et al. (2001), who found that thermal treatment leads to structural modifications and the generation of new molecules in seed oils.

The antioxidant analysis revealed that the oil had moderate free radical scavenging activity (62%), which was less than that of the synthetic antioxidant Propyl Gallate. This level of activity is consistent with findings by Rowland et al. (2017), who noted that natural oils, though not as potent as synthetic antioxidants, still offer protection against oxidative stress. The DSC data further reinforced the oil's thermal stability, with melting point analysis showing resilience up to 150°C, a useful trait for cosmetics and food applications, as confirmed by Michalak et al. (2024).

Although the antimicrobial tests showed limited inhibition of Staphylococcus aureus, with no efficacy against Escherichia coli or Candida albicans, this result aligns with Man et al. (2019), who noted that Buckthorn oil's antimicrobial potential might be limited and could require combination with stronger agents.

## 5. Conclusions

The analysis of Buckthorn seed oil demonstrated considerable shifts in chemical composition post-heating, indicating that thermal processing induces notable changes in its molecular makeup. The oil's moderate antioxidant activity, while not as effective as synthetic antioxidants like Propyl Gallate, shows potential for use in natural formulations. However, the oil's antimicrobial effectiveness was weak, with minimal activity against Staphylococcus aureus and no observed activity against Escherichia coli and Candida albicans. Despite this, the oil's thermal stability, as confirmed by DSC analysis, makes it a viable candidate for cosmetic and food industry applications where moderate heat resistance is required. These findings suggest that further optimization, particularly in enhancing its bioactive properties, could broaden the oil's applicability in different industries.

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## Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relation-nships that could have appeared to influence the work reported in this paper.

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