Characterization of Buckthorn Seed Oil: Composition, Thermal Properties, and Bioactive Potential

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Abstract

This study focused on the chemical composition, thermal properties, antioxidant, and antimicrobial activities of buckthorn seed oil. The GC/MS analysis identified twelve compounds, with cis-Vaccenic acid (20.39%) and 9,12-Octadecadienoic acid (Z,Z) (18.71%) as dominant constituents. Heating caused significant transformations, including the formation of Trilinolein (20.77%). Antioxidant activity, measured using the DPPH method, showed a moderate % Radical Scavenging Activity (%RSA) of 70.5%, compared to 90.5% for the synthetic control, Propyl Gallate. Antimicrobial tests indicated low sensitivity against Staphylococcus aureus and resistance to Escherichia coli and Candida albicans, suggesting limited antimicrobial efficacy. DSC analysis revealed a melting point near 40°C and thermal stability up to 150°C, followed by additional endothermic transitions. These findings suggest that buckthorn seed oil has promising applications in food processing, cosmetics, and pharmaceuticals, although its antimicrobial activity may require further enhancement for broader use.

Keyword: *Buckthorn seed oil, GC/MS analysis, Bioactivity, DSC thermal analysis*

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 [1]. 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 [2]. These bioactive compounds make buckthorn seed oil a promising ingredient in health and cosmetic products, offering antioxidant, anti-inflammatory, and antimicrobial benefits (3). 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 [3-4].

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 [5]. It also contains significant amounts of phytosterols like beta-sitosterol, which is known for lowering cholesterol levels and providing cardioprotective effects [6]. These attributes make the oil especially beneficial in skincare formulations aimed at improving skin health and combating oxidative stress [7]. 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 [8].

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 [9]. 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 [10]. 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 [11]. 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 [12].

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 [13]. 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 [14]. Understanding the oil's melting and crystallization points can help optimize formulations that undergo heat processing, such as emulsions and creams [15].

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 [16]. 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 [17].

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 [18]. 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. Methodology

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 [19].

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 [20].

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 [21].

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 [22].

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 [23].

3. Results and discussion

3.1 Chemical Composition Analysis for buckthorn oil sample:

3.1.1 Before heating:

The GC/MS analysis of buckthorn seed oil revealed twelve bioactive compounds, collectively accounting for 100% of the total fraction. The major component was cis-Vaccenic acid (20.39%), which is known for its cardiovascular health benefits and contributions to skin health. Linoleic acid, present at 18.71%, is well recognized for its ability to support skin hydration and maintain the skin barrier. Gamma-Sitosterol (12.78%) and stigmasterol, two significant phytosterols in the oil, possess anti-inflammatory and cholesterol-lowering properties, further supporting the use of buckthorn oil in nutraceutical and cosmetic formulations. Additionally, gamma-tocopherol (11.5%) and delta-tocopherol (3.41%) contribute antioxidant activity, helping to prevent oxidative stress and lipid peroxidation—key factors in aging. Nonanal (2.57%) adds sensory appeal, potentially making buckthorn oil more marketable in cosmetics [24-27].

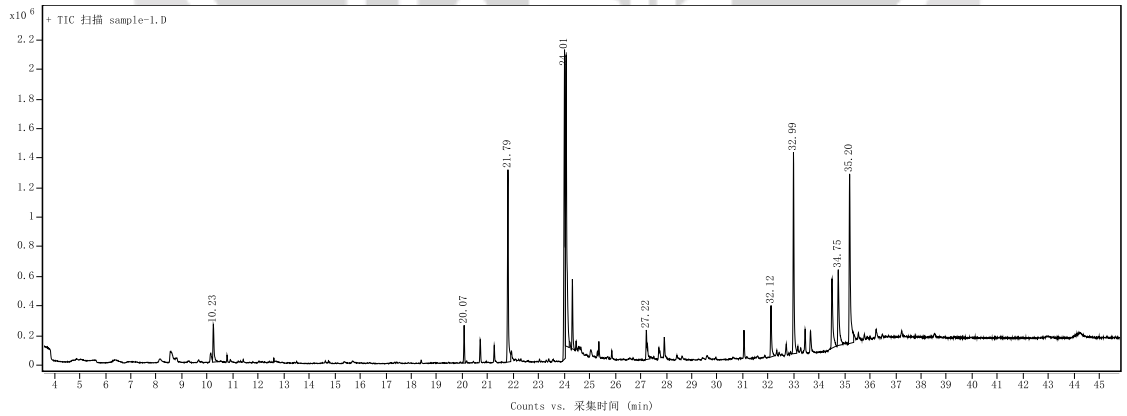

Figure 1: *GC/MS chromatogram of components in buckhorn oil before heating*

Table 1: Major chemical constituents of buckthorn oil before heating:

3.1.2 After heating:

No	Ret.time	Compound	M.F	M.W g/mol	%	Area %
1	10.23	Nonanal	C9H18O	142.24	2.57	12.58
2	20.07	Neophytadiene	C20H38	278.48	1.67	8.17
3	21.79	n-Hexadecanoic acid	C16H32O2	256.42	11.45	56.13
4	24.01	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280.45	18.71	91.74
5	24.08	cis-Vaccenic acid	C18H34O2	282.46	20.39	100
6	24.32	Octadecanoic acid	C18H36O2	284.48	3.36	16.47
7	27.22	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C21H38O4	354.50	2.33	11.41
8	32.12	.delta.-Tocopherol	C27H46O2	402.65	3.41	16.73
9	32.99	.gamma.-Tocopherol	C28H48O2	416.68	11.5	56.41
10	34.51	Campesterol	C28H48O	400.68	5.82	28.54
11	34.75	Stigmasterol	C29H48O	412.69	6.03	29.56
12	35.2	.gamma.-Sitosterol	C29H50O	414.71	12.78	62.67

Post-heating analysis of buckthorn seed oil revealed significant changes in its chemical composition. Trilinolein (20.77%) became the most abundant compound, signaling possible chemical processes such as esterification or polymerization. Linoleic acid, now measured at 17.84%, underwent transformations, as evidenced by the formation of esters such as 9 Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester (14.67%). Gamma-tocopherol (5.92%) and Vitamin E (3.44%) showed some degradation but were still present, indicating that the oil retained part of its antioxidant properties even after thermal treatment. Sterols such as gamma-Sitosterol (5.85%), stigmasterol (2.56%), and campesterol (2.28%) also persisted, maintaining their beneficial health properties. However, the formation of nonanal (2.57%) highlighted oxidative changes that occurred due to heating [28-33].

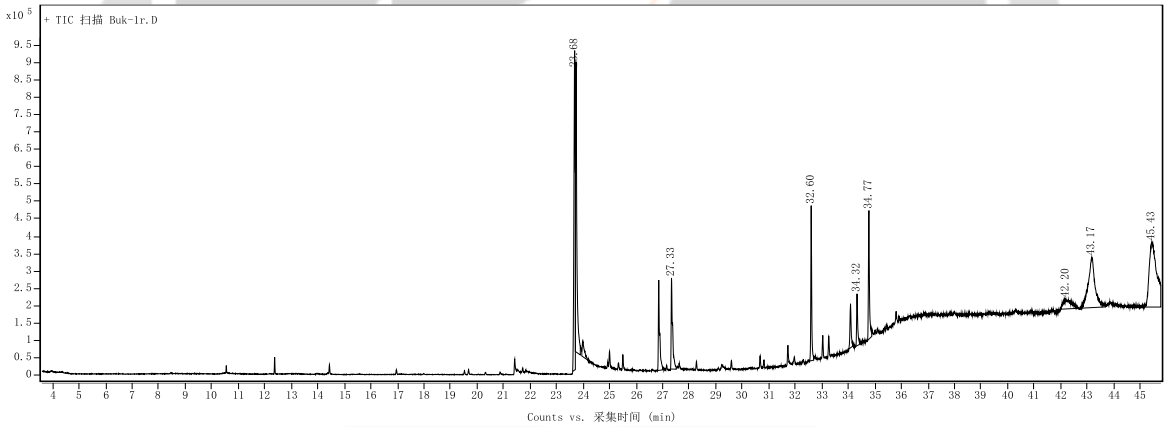

Figure 2: *GC/MS chromatogram of components in buckthorn oil after heating*

Table 2: Chemical Composition of buckthorn seed oils After Heating:

No	Ret. time	Compound	M.F	M.W g/mol	%	Area %
1	23.68	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	16.39	78.91
2	23.73	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46	16.38	78.83
3	23.98	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	1.45	6.97
4	26.85	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354.50	4.84	23.32

5	27.33	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354.50	5.44	26.18
6	32.6	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	416.68	5.92	28.51
7	34.08	Campesterol	$C_{28}H_{48}O$	400.68	2.28	10.98
8	34.32	Stigmasterol	$C_{29}H_{48}O$	412.69	2.56	12.33
9	34.77	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.71	5.85	28.17
10	42.2	Vitamin E	$C_{29}H_{50}O_2$	430.71	3.44	16.57
11	43.17	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	$C_{37}H_{70}O_5$	614.97	14.67	70.62
12	45.43	Trilinolein	$C_{57}H_{98}O_6$	884.43	20.77	100

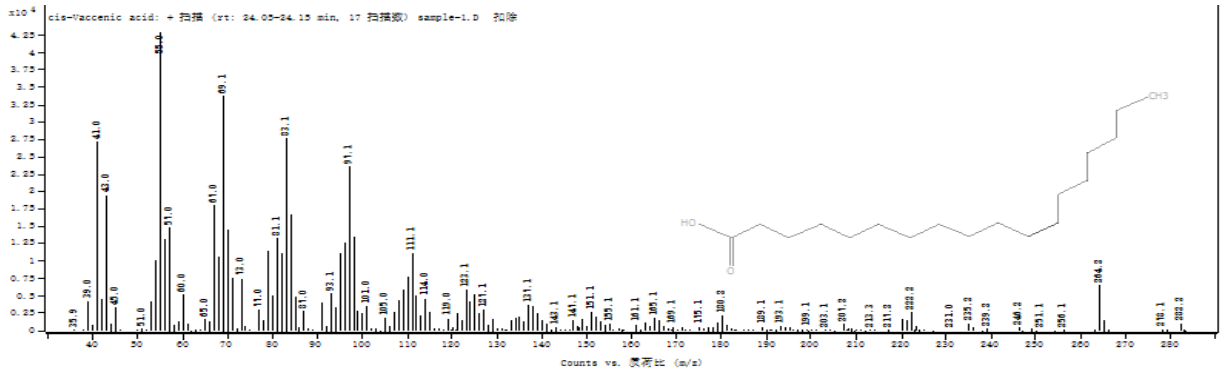

***Figure 3**: Mass spectrum of 11-Octadecenoic acid, (Z)- (cis-Vaccenic acid)*

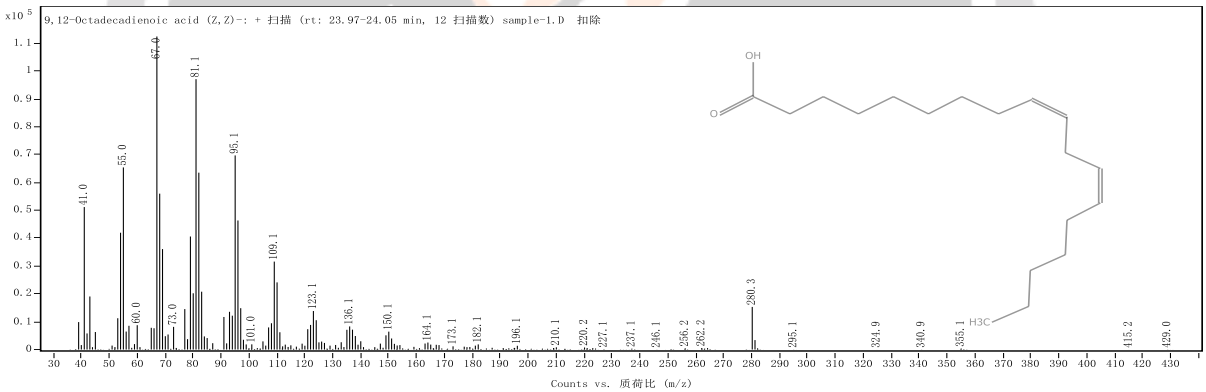

***Figure 4**: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-*

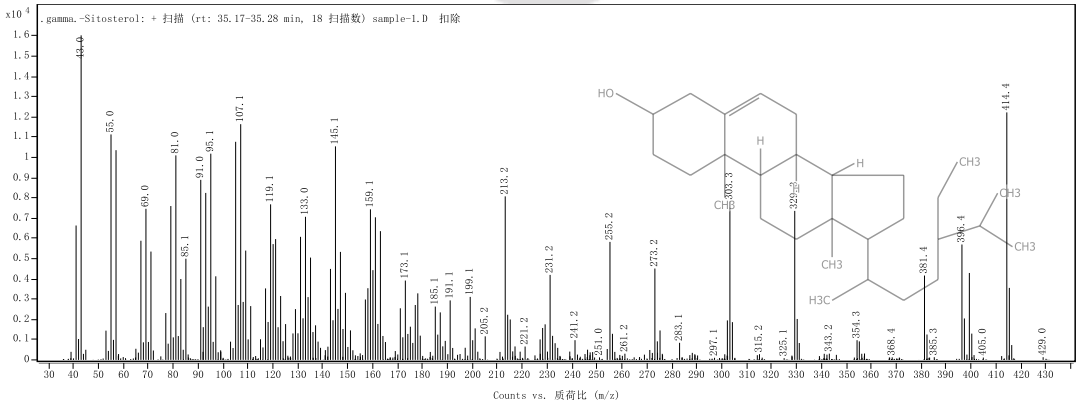

***Figure 5**: Mass spectrum of Gamma. -Sitosterol*

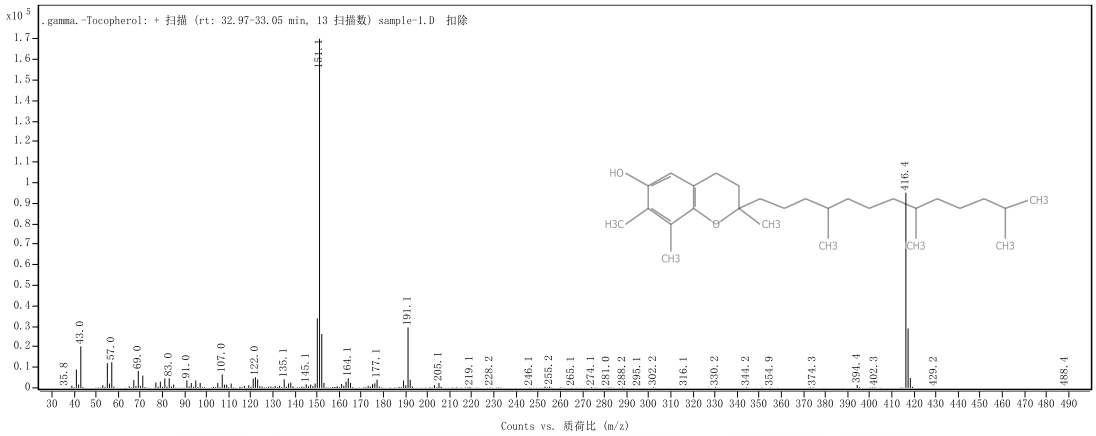

***Figure 6**: Mass spectrum of Gamma. -Tocopherol*

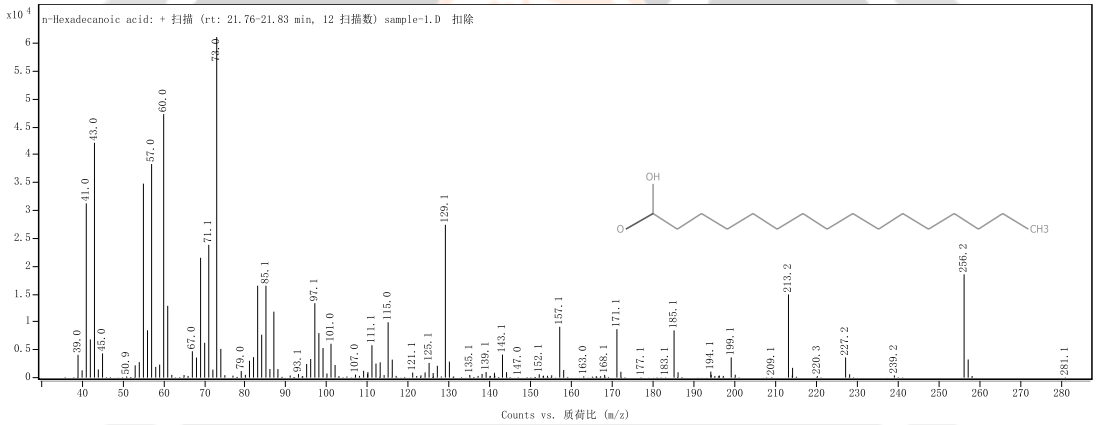

***Figure 7**: Mass spectrum of n Hexadecanoic acid*

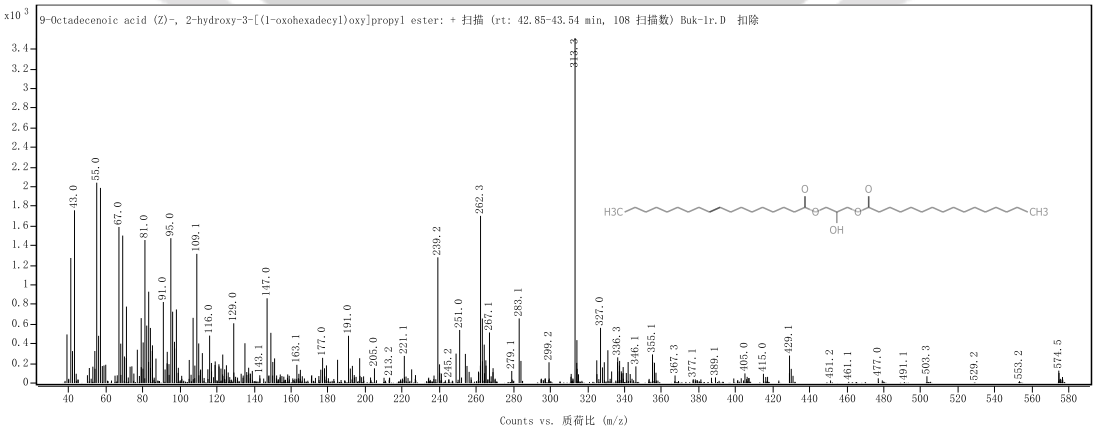

***Figure 8**: Mass spectrum of* 9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester

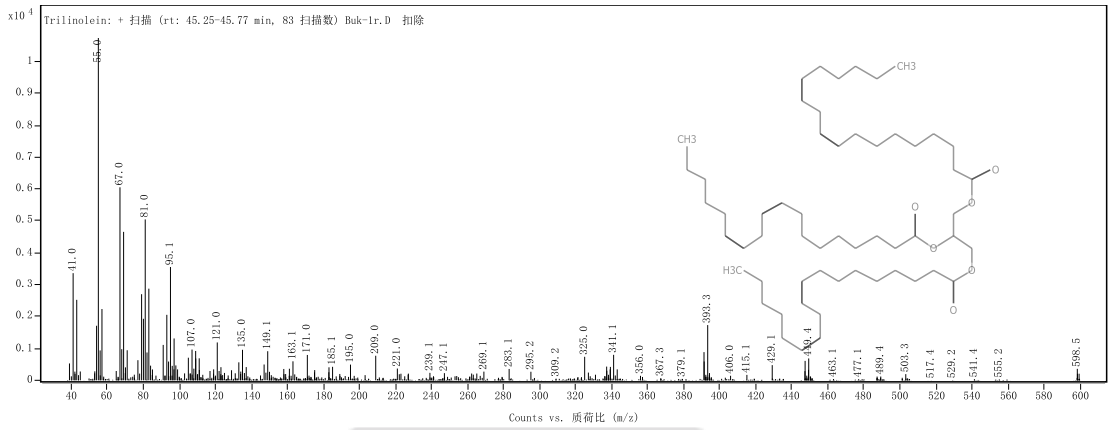

Figure 9: *Mass spectrum of Trilinolein*

Table 3: template for comparing the GC/MS results of buckthorn seed oil before and after heating:

Component Name	Before H (Presence/ Absence)	After H (Presence/ Absence)	% Change	Notes
9,12-Octadecadienoic acid (Linoleic acid)	Present	Present	Decreased from 18.71% to 17.84%	Slight reduction; suggests partial breakdown or transformation
cis-Vaccenic acid	Present	Present	Decreased from 20.39% to 16.38%	Noticeable decline; maybe due to thermal degradation
gamma-Tocopherol (Gamma-tocopherol)	Present	Present	Decreased from 11.5% to 5.92%	Significant loss owing to sensitivity to heat
gamma-Sitosterol	Present	Present	Decreased from 12.78% to 5.85%	Major reduction; signifies substantial thermal instability
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Present	Present	Increased from 2.33% to 10.28%	Significant increase; suggests creation from breakdown of other components
Stigmasterol	Present	Present	Decreased from 6.03% to 2.56%	Reduction owing to heat, indicating moderate thermal instability
Campesterol	Present	Present	Decreased from 5.82% to 2.28%	Substantial drop; demonstrates vulnerability to heat
delta-Tocopherol	Present	Absent	Not detected	Completely deteriorated in hot circumstances
Octadecanoic acid	Present	Absent	Not detected	Disappears after heating, indicating full breakdown
Neophytadiene	Present	Absent	Not detected	Not detected after heating, perhaps due to thermal degradation
Nonanal	Present	Absent	Not detected	Not found post-heating; likely evaporated or disintegrated
n-Hexadecanoic acid (Palmitic acid)	Present	Absent	Not detected	Completely destroyed or altered due to heat exposure

9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl)oxy]propyl ester	Not present	Present	N/A	Newly synthesized compound likely originating from heat reactions
Trilinolein	Not present	Present	N/A	Newly generated compound possibly due to complicated esterification reactions
Vitamin E (Tocopherol)	Not present	Present	N/A	Newly created compound; suggests conversion or reaction involving existing components

Impact of Thermal Processing on Bioactive Stability:
Thermal processing led to considerable shifts in buckthorn seed oil's chemical composition. Cis-Vaccenic acid decreased from 20.39% to 16.38%, and there was an increase in the formation of 9,12-Octadecadienoic acid esters, indicating transformations of unsaturated fatty acids during heating. Gamma-Sitosterol experienced a reduction from 12.78% to 5.85%, and the appearance of nonanal (2.57%) suggested oxidative degradation. Although gamma-tocopherol and Vitamin E levels declined after heating, they remained present, reflecting partial retention of antioxidant properties. These findings underscore the effects of heating on buckthorn oil's chemical profile, with the formation of new esters and varying degrees of stability in its bioactive compounds.

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	70.5% ± 1.9 µg/mL
Propyl Gallate (Standard)	90.5% ± 0.8 µg/mL

The antioxidant potential of buckthorn seed oil was evaluated using the DPPH radical scavenging method, yielding a % Radical Scavenging Activity (%RSA) of 70.5% ± 1.9 µg/mL. Although this activity is moderate when compared to the control, Propyl Gallate, which displayed a significantly higher %RSA of 90.5% ± 0.8 µg/mL, buckthorn oil still demonstrates considerable antioxidant capacity. Its free radical scavenging ability, likely due to bioactive compounds like tocopherols and phytosterols, indicates its potential as a natural antioxidant source. Although less effective than synthetic antioxidants, buckthorn oil can be valuable in preventing oxidative damage, particularly in the food and cosmetic industries, where natural antioxidants are increasingly sought after. With further optimization or in combination with other antioxidants, the effectiveness of buckthorn oil could be enhanced for broader applications.

3.4 Explanation of DSC Results for Buckhorn Sample:

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.

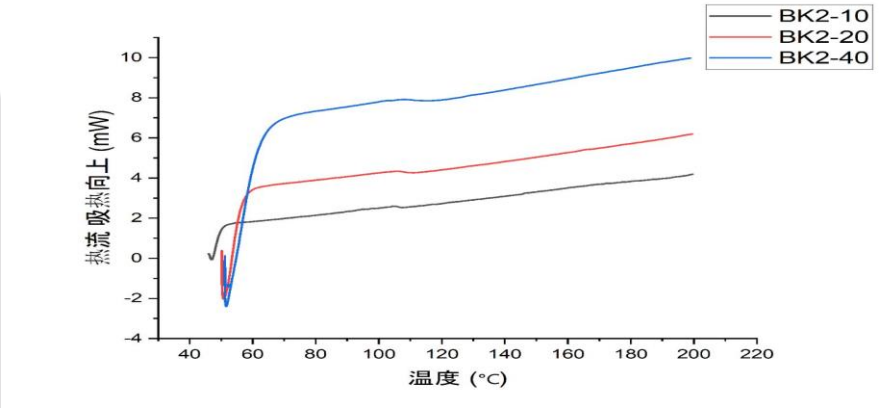

Figure 10: DSC Thermograms for Buckhorn oil

4. CONCLUSION

A detailed assessment of buckthorn seed oil's chemical and bioactive properties, alongside its thermal behavior, highlights its potential for various applications. The GC/MS results identified key compounds like cis-Vaccenic acid, 9,12-Octadecadienoic acid (Z,Z), and gamma-Sitosterol. Notable transformations post-heating, including the emergence of Trilinolein and other esters, illustrate the oil's sensitivity to thermal changes. Despite these modifications, bioactive compounds such as gamma-Tocopherol persisted, contributing to the oil's moderate antioxidant activity, reflected by its % Radical Scavenging Activity (%RSA) of 70.5%, though this was lower than the synthetic control, Propyl Gallate. Antimicrobial activity tests revealed limited efficacy, with minor sensitivity against *Staphylococcus aureus* and resistance to *Escherichia coli* and *Candida albicans*, suggesting that improvements or combinations with other agents may be needed to enhance its antimicrobial properties. Additionally, DSC analysis indicated a melting point near 40°C and stability up to 150°C, positioning buckthorn oil as suitable for thermal processes in industries like food, cosmetics, and pharmaceuticals. These comprehensive results point to the oil's utility, although its full potential hinges on better leveraging its bioactive components and improving its antimicrobial activity.

5. ACKNOWLEDGMENT

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