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Analysis of Fatty Acids in Wool Oil Extracted from Mongolian Sheep Wool using Gas Chromatography and Infrared Spectrophotometry

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ABSTRACT

To develop a method for extracting wool oil from Mongolian sheep wool, evaluate its quality, and determine the fatty acid composition. The extraction of wool oil was performed using the Soxhlet method. Fatty acid content was determined by gas chromatography, while saponification and ester numbers were assessed using titrimetric methods. Functional compounds in the wool oil were identified using infrared spectrophotometry, with further analysis conducted through gas chromatography. Infrared spectroscopy identified valence and deformation oscillations at 3308.01 cm-1, 2922.47 cm-1, 1743.46 cm-1, 1378.34 cm-1, 1465.76 cm-1, 720.83 cm-1, and 1085.39 cm-1. Gas chromatography revealed 14 distinct fatty acids, with saturated fatty acids accounting for 66.6%, and unsaturated fatty acids making up 33.3%. Polyunsaturated fatty acids comprised 26.26%, while monounsaturated fatty acids represented 7.14% of the total. The analysis identified key functional groups (C=O, CH3, CH2, C-C, C-O, O-H) in wool oil using infrared spectroscopy. The fatty acid composition of the extracted wool oil included a predominance of saturated fatty acids (66.6%) and a significant proportion of polyunsaturated fatty acids (26.26%). This method provides a viable alternative for extracting and utilizing wool oil from Mongolian sheep wool.

Keywords: Functional groups; Lanolin; Saponification; Soxhlet extraction

INTRODUCTION

This study investigates the extraction and quality evaluation of wool oil from Mongolian sheep wool, aiming to develop a domestic lanolin production process. Mongolian sheep wool, historically referred to as "White Gold," is valued for its hygienic properties and is traditionally used in the production of various goods such as clothing, carpets, and housing materials. Despite the large number of sheep in Mongolia, a significant portion of the wool remains untreated due to the lack of local processing capabilities. The extraction of lanolin, a substance rich in fatty acids and functional groups, could offer significant benefits by reducing reliance on imported raw materials[1].

According to the latest data from international trade statistics, Mongolia produced over 30,000 tons of raw sheep wool annually, and in 2023 exported wool worth approximately USD 363 million[2]. Despite this large raw material base, Mongolia continues to import refined lanolin, with market surveys indicating an import volume of approximately 5 tons per year at a cost ranging from USD 40,000 to 60,000 depending on purity and origin[1]. This reliance on imports underscores a critical gap in domestic processing infrastructure.

The composition of sheep wool includes 80% keratin, 17% non-protein compounds, and 1% lipid, with 5-25% of the wool's weight being lanolin. This oil is especially valuable due to its high content of long-chain fatty acids, including oleic and linoleic acid, which resemble the natural oils produced by human skin, providing effective moisturizing and skin-soothing benefits[1]. The nutritional status of the sheep, influenced by their grazing environment, affects the fatty acid profile of the wool oil. Pastured sheep tend to have wool oils richer in omega-3 fatty acids, while those fed grain-based supplements have oils higher in saturated fatty acids.

This study will focus on the Soxhlet extraction method for obtaining wool oil from Mongolian sheep wool and utilize infrared spectroscopy and gas chromatography to analyze the oil's fatty acid content and quality. The research aims to establish the feasibility of lanolin production from locally sourced wool, with the goal of fostering industrial-scale production. The results will not only help in reducing Mongolia's dependence on imported lanolin but also promote the creation of natural, cost-effective products. The novelty of this research lies in the development of an extraction process tailored to Mongolia's specific sheep wool characteristics.

By developing indigenous lanolin extraction capabilities, Mongolia stands to reduce foreign dependence and retain a greater proportion of value within the national economy. Such advancements would not only reduce production costs for domestic cosmetic and pharmaceutical industries but also generate new economic opportunities in rural areas, where raw wool is sourced. Encouraging local processing facilities would strengthen the supply chain, promote employment in herding communities, and contribute to sustainable economic development[1,3].

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By achieving these goals, the study will contribute to the practical application of lanolin extraction technology in Mongolia, opening doors to local manufacturing and supporting economic growth in the region.

MATERIAL AND METHODS

Raw Material Collection: In this study, raw sheep wool samples were collected from Duut, Chandman, and Dundgov provinces, along with samples from the Dashbalbar sum (Buriad breed) in Dornod province. The collection occurred during the period of June 15 to July 5 in both 2020 and 2021. The study was supported by the School of Pharmacy at the Mongolian National University of Medical Sciences, the Laboratory of Drug Analysis under the Department of Control and Regulation of Medicines and Medical Devices, and the Institute of Chemistry and Chemical Technology at the Mongolian Academy of Sciences.

Oil Extraction Method: The extraction of sheep wool oil was carried out using the Soxhlet extraction method. Wool was first cleaned by removing fine dirt, vermin, and lichens, and was then classified into native wool and primary wool. The wool was dried thoroughly before being wrapped in filter paper and placed in a Soxhlet extractor. Solvent was added until the sample was completely covered, and the system was connected to a rotary cooler. The temperature was adjusted to 50-60°C, and extraction was carried out for 1-3 hours. After extraction, the solvent was evaporated at 40-70°C, and the oil was dried in a flask at 105°C to obtain a constant weight. The weight of the oil was calculated using the formula:

$\beta=(a-b)/c\times100$

Where:

- a = Weight of flask with oil (G)
- b = Weight of flask without oil (G)
- c = Weight of sample taken for analysis (G)
- β = Amount of oil contained in 100 G of wool (percentage)

Functional Group Determination by Infrared Spectrophotometry: To identify the functional compounds, present in the extracted wool oil, an infrared spectrophotometer (FTIR Nicolet iS-10 Spectrometer with a Zinc Selenide ATR accessory, Thermo Fisher Scientific, USA) was employed. Samples were analyzed in the frequency range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. A blank background spectrum was collected before each analysis to attain a relative scale for absorption intensity. Each sample was run three times for optimization. After each run, the ATR crystal was cleaned using 70% isopropanol to ensure accurate results.

Gas Chromatography Method for Fatty Acid Analysis

Gas chromatography was used to analyze the fatty acid content in the wool oil. Fatty acid methyl esters were prepared by dissolving 20 mg/ml of the sample in chemically pure hexane. Nonadecanoic acid (C19:0, $CH_3(CH_2)_{17}COOH$) was used as an internal standard. Fatty acid composition was determined by comparing the sample with fatty acid methyl esters mix (Sigma-Aldrich, CRM 18918) as the standard. The gas chromatography analysis was performed using an Agilent 7980 instrument equipped with a Restek HP-88 column (length 60mm, diameter 0.25mm, thickness 0.2μm). The column temperature was initially set at 120°C and gradually increased to 250°C. Nitrogen was used as the carrier gas, and the pump and detector temperatures were maintained at 250°C. A 1 μL injection volume was used, and a 30:1 compression ratio was maintained throughout the analysis.

Sample Preparation for Gas Chromatography: Wool oil was suspended in water and extracted three times with hexane to remove oil and particulate matter. The wool oil was then methylated by dissolving 50 mg of the oil in 1 mL of benzene and adding 2 mL of 5% hydrochloric acid methanol solution. The mixture was boiled for 2 hours in a rotary evaporator. After methylation, 5 mL of 5% KCl solution was added, and the methyl esters were extracted with hexane (2-3 times in a separatory funnel). The hexane phases were combined, filtered through anhydrous sodium sulfate to remove water, and then concentrated in a vacuum evaporator at 40°C to remove any residual solvent.

Fatty Acid Composition Analysis: The fatty acid methyl esters prepared from wool oil were analyzed by gas chromatography using an Agilent 7980 instrument. The fatty acid composition was determined by comparing the results with fatty acid methyl esters mix (Sigma-Aldrich, CRM 18918) as a reference standard.

RESULTS AND DISCUSSION

Wool Oil Extraction Results Using the Soxhlet Method: To determine the optimal extraction time and solvent for raw wool oil extraction, raw wool samples were prepared, evaluated for quality, and measured for length. The extraction was conducted at temperatures between 50°C and 60°C using different solvents for durations of 1, 2, and 3 hours. The extract was then concentrated at temperatures ranging from 40°C to 70°C to obtain the wool oil.

Selection of the Appropriate Solvent for Wool Oil Extraction: The yield of wool oil extracted using three different organic solvents was compared, and a one-way analysis of variance (ANOVA) revealed a statistically significant difference ($p = 0.0001$) between the solvents. At 1, 2, and 3 hours of extraction, all solvents (diethyl ether: ethanol, diethyl ether: acetone-hexane mixture, and ethanol: acetone-hexane mixture) exhibited statistically significant differences ($p = 0.0001$), with ethanol showing the highest oil content in comparison to the other solvents. The highest oil content was obtained using ethanol. (Figure 1)

Selection of the Optimal Time for Extracting Wool Oil: A one-way repeated measures ANOVA was conducted to assess variations in wool oil yield extracted using different organic solvents over 1 hour, 2 hours, and 3 hours. The highest oil content was observed in samples extracted with ethanol for 3 hours, with statistically significant differences ($p = 0.0001$), indicating that the optimal extraction time is 3 hours. Oil content decreased when the extraction time exceeded 3 hours. (Figure 2)

Impact of Fresh and Stored Wool on Oil Yield: We determined the total oil content of six samples of freshly sheared sheep wool and stored (1-year storage) wool from Dundgobi, Khovd, and Dornod provinces, representing three different regions, by Soxhlet extraction for 3 hours. The total oil content of freshly sheared and stored (1-year storage) wool from Dundgobi, Khovd, and Dornod provinces was determined by Soxhlet extraction for 3 hours. The highest oil yield was found in freshly sheared wool from Dundgobi (9.77% ± 0.58), followed by Dornod (7.37%), and the lowest yield was observed in stored wool from Khovd (6.46% ± 0.32). As shown in Figure 3, the oil yield from freshly sheared wool was significantly higher ($p = 0.004$) compared to stored wool (Figure 3).

Furthermore, in subsequent investigations, we conducted a detailed analysis of the quality of wool oil obtained by extracting freshly sheared sheep wool from Dundgobi province using 99.6% ethanol.

Results of Analysis of the Fatty Acid Composition of Wool Oil

Analysis of Functional Groups' Absorption in Wool Oil: Infrared spectroscopy was used to analyze the functional groups in wool oil. Absorption bands corresponding to C-O, CH, C=O, and OH groups were detected. (Figure 4).

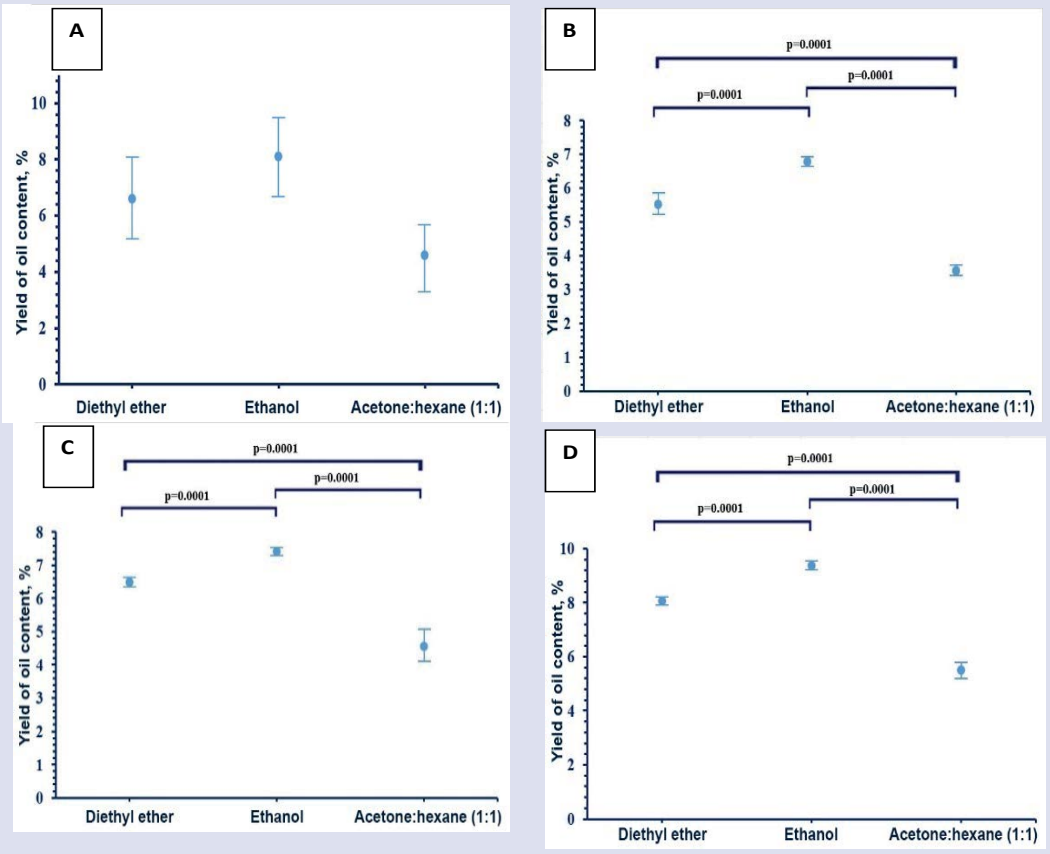

Figure 1. Yiels of oil content the studied wool oils**. (A)** Comparison of organic solvents; As the p-value was below 0.05, a 2 by 2 comparative post-hoc analysis was conducted (**B)** Comparison of oil content extracted with organic solvents for 1 hours.; **(C)** Comparison of oil content extracted with organic solvents for 2 hours; **(D)** Comparison of oil content extracted with organic solvents for 3 hours.

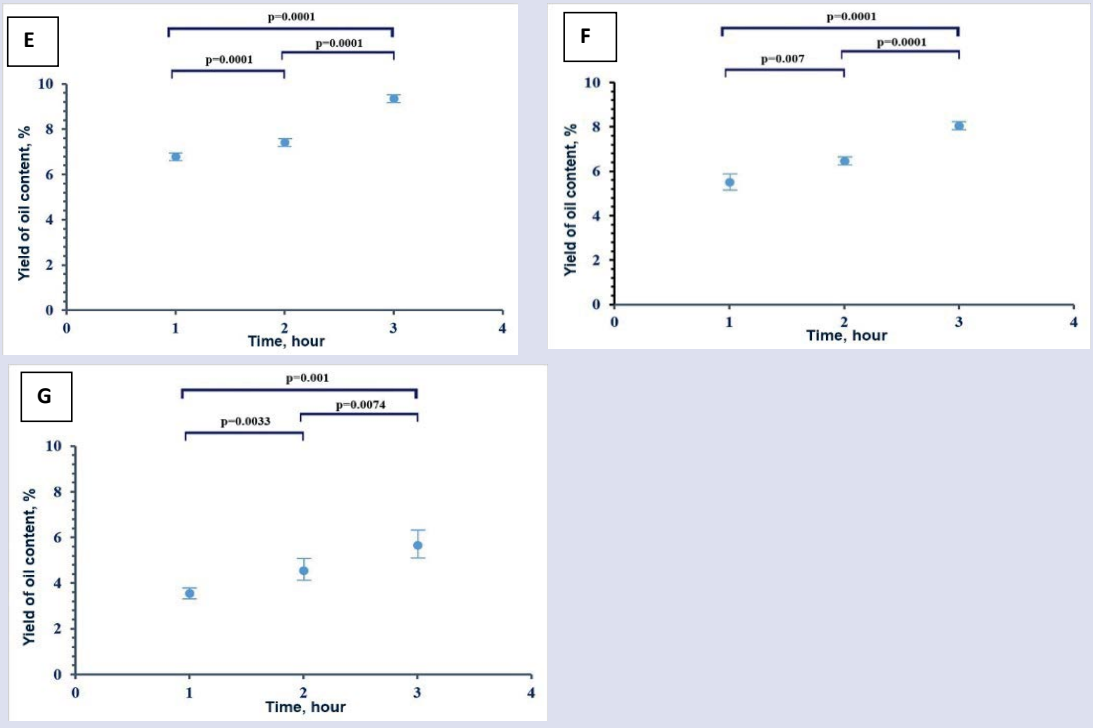

Figure 2. Selection of the Optimal Time for Extracting Wool Oil; **(E)** Time dependence of oil content extracted using ethanol; **(F**) Time dependence of oil content extracted using diethyl ether; **(G)** Time dependence of oil content extracted using 1:1 mixture of acetone: hexane.

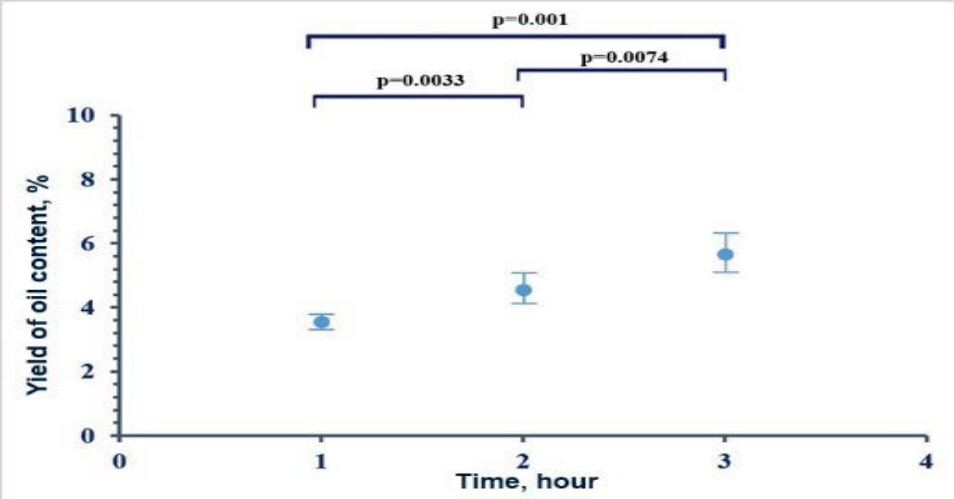

Figure 3. Comparison of wool oil yield between fresh and stored wool.

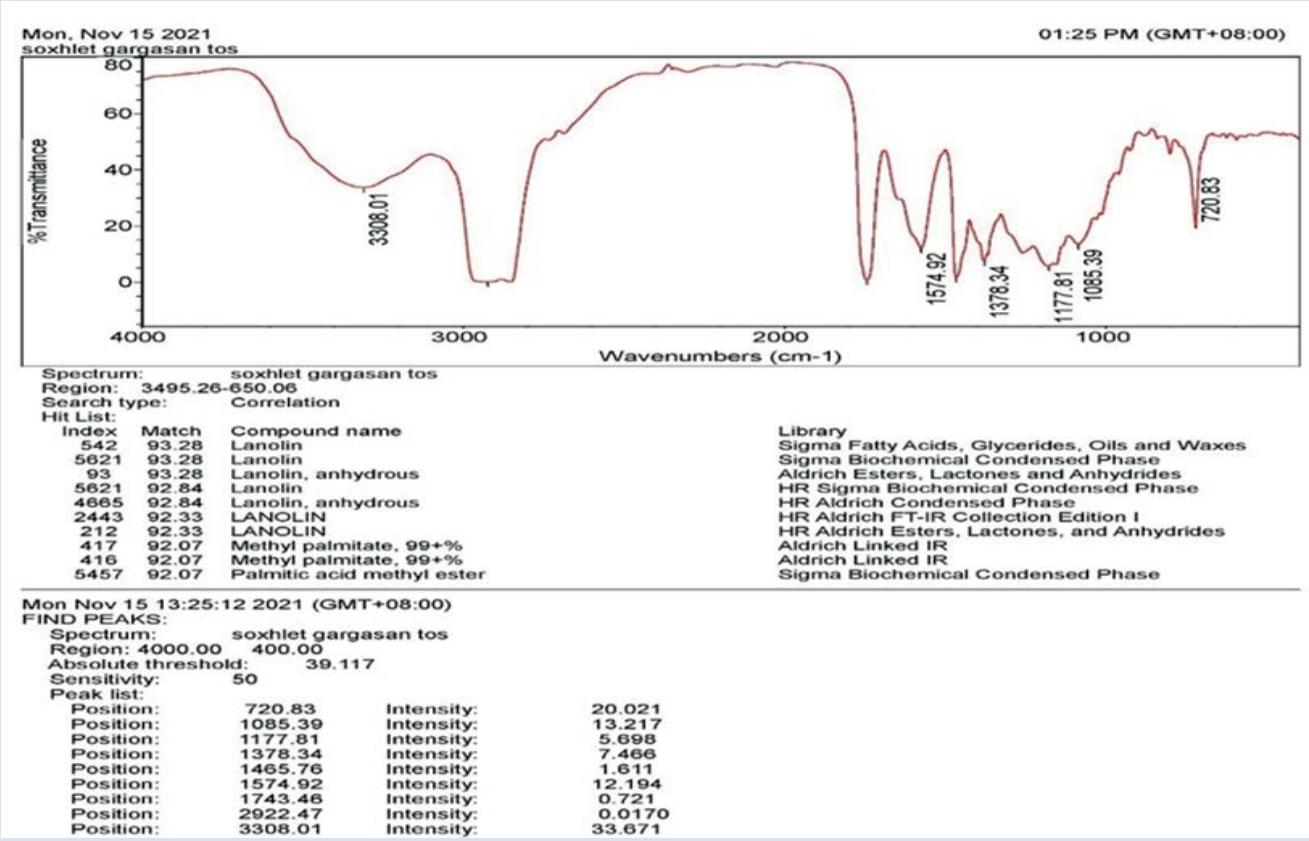

Figure 4. Oil Extraction Spectrum.

The infrared spectrum revealed the following absorption bands:

IR spectrum, ν max, cm –1: 3308.01 (O-H), 2922.47 (C-H in CH3), 1743.46 (C=O), 1378.34 (C-H in CH3), 1465.76 (C-H in CH2), 1177.81 (C-O), 720.83 (C-C).2.2 Analysis of Fatty Acid Composition Using Gas Chromatography

The fatty acid composition of sheep wool oil was analyzed using a set of 14 fatty acid standards. (Table 1)

The analysis detected all 14 fatty acids, with saturated fatty acids dominating (66.6%), especially palmitic acid (23.52%), stearic acid (22.23%), and myristic acid (9.27%). Unsaturated fatty acids accounted for 33.4%, with oleic acid being the most abundant at 13.66%, followed by palmitoleic acid (9.11%) and erucic acid (3.49%). (Figure 5)

Quality Parameters: Fatty Acid Content, Saponification Value, and Iodine Value The number of fatty acids, saponification value, and iodine value were determined and summarized in Table 2. The wool oil exhibited an average fatty acid amount of 5.054 ± 0.04 mg, a saponification value of 82.69 ± 0.34 mg, and an iodine value of 74.80 ± 0.41 mg (n = 5).

Table 1. Fatty Acid Composition of Wool Oil Extracted from Mongolian Sheep Wool

Fatty Acid	Label	Content (%)
Caprylic acid	C8:0	0.30
Capric acid	C10:0	0.57
Lauric acid	C12:0	1.48
Myristic acid	C14:0	9.27
Palmitic acid	C16:0	23.52
Palmitoleic acid	C16:1	9.11
Stearic acid	C18:0	22.23
Oleic acid	C18:1	13.66
Linoleic acid	C18:2	0.71
Linolenic acid	C18:3	6.43
Arachinic acid	C20:0	3.64
Benzene acid	C22:0	3.13
Erucic acid	C22:1	3.49
Lignoceric acid	C24:0	2.45

Table 2. Wool Oil Quality Parameters.

Indicator	Frequency (n = 5)	Mean ± SD
Number of Fatty Acids (mg)	5.05, 5.0, 5.07, 5.1, 5.05	5.054 ± 0.04
Saponification Value (mg)	82.18, 83.04, 82.95, 82.72, 82.56	82.69 ± 0.34
Iodine Value (mg)	74.38, 74.7, 75.12, 75.33, 74.49	74.80 ± 0.41

DISCUSSION

Mongolia's sheep population has seen steady growth in recent years, as reflected in the national livestock census, with a 5.3% increase over the previous year[3]. This consistent rise underscores the importance of effectively utilizing the abundant animal resources available in a pastoral nation like Mongolia. Efficient technological processing of Mongolian sheep's wool, including the extraction of wool oil, is vital for developing local industries that can substitute imports, expand pharmaceutical excipient production, and cater to the growing demand for natural products[1]. In this context, optimizing wool oil extraction methods and improving wool oil quality is essential for future industrial applications[4].

Recent global reports indicate that the lanolin market has experienced steady growth due to increasing demand from the cosmetics, pharmaceuticals, and personal care industries. According to a comprehensive market analysis by Grand View Research, the global lanolin market was valued at USD 415 million in 2022 and is projected to grow at a compound annual growth rate of 5.8% from 2023 to 2030[5] The demand is driven by lanolin's unique moisturizing and protective properties, making it an essential ingredient in skin care formulations worldwide. Major producers of lanolin include Australia, New Zealand, and China, with Australia alone accounting for nearly 50% of global lanolin exports[6]. Despite Mongolia's significant sheep population and raw wool production, its participation in global lanolin markets remains minimal, primarily due to limited processing infrastructure and export capacity.

Historically, wool wax (or lanolin) has been extracted from sheep's wool for millennia, with the earliest known use being in the 7th century BC[7]. Today, wool oil is extracted using elaborate industrial methods, such as centrifugation in washing plants, but these techniques are costly[8]. To address this, our study employed the Soxhlet method using organic solvents under laboratory conditions, an approach that offers a more accessible and efficient means of wool oil extraction[9].

Our study aimed to improve the extraction of wool oil from Mongolian sheep wool, focusing on determining the most effective solvent and extraction duration. In comparison to past studies, such as those by Ghidaoui and Aissani (2022) and Choijil and Bazarragas chromatographyhaa (1998), we utilized ethanol, diethyl ether, acetone, and hexane to extract wool oil from male sheep wool[10]. The findings indicated that ethanol (99.6%) provided the highest oil yield (9.36% ± 0.09), which was significantly higher than that obtained with the other solvents[1]. This result is consistent with the hypothesis that ethanol, being a highly polar solvent, is more effective in extracting wool oil compared to less polar solvents like hexane[11]. Ethanol presents several notable advantages over conventional solvents such as diethyl ether, acetone, and hexane, particularly due to its low toxicity and greener processing profile. It's low-toxicity and renewable bio-based origin make it suitable for use in products intended for human use, including cosmetics and pharmaceuticals[12,13]. In contrast, solvents like hexane and ether are associated with higher toxicity and environmental hazards[14,15]. Overall, ethanol supports a safer, cleaner and more sustainable lanolin extraction process. Notably, our study also confirmed that fresh wool yielded higher oil content than stored wool, emphasizing the importance of using freshly sheared wool for optimal extraction[16].

The findings of previous studies, such as those by Makrem Ghidaoui and Nadhem Aissani (2022), who reported an oil yield of 20% using hexane, showed that yields can vary significantly depending on the method and solvent used. In comparison, our Soxhlet extraction method yielded wool oil ranging from 6.03% to 10.6%, depending on the solvent[17]. The variation observed in these results can be attributed to several factors, such as the composition of the wool and the solvent's ability to solvate lanolin[4].

The analysis of the fatty acid composition in wool oil revealed high concentrations of saturated fatty acids, including palmitic acid (23.52%), stearic acid (22.23%), and myristic acid (9.27%), which have known applications in cosmetic and pharmaceutical formulations[18]. Furthermore, unsaturated fatty acids such as oleic acid (13.66%), palmitoleic acid (9.11%), and linolenic acid (6.43%) were present in significant quantities[19]. These fatty acids are crucial for various health applications, with oleic acid recognized for its cardiovascular benefits and linolenic acid (an omega-3 fatty acid) being vital for overall well-being[20]. The results align with studies suggesting that lanolin and its derivatives, with their rich fatty acid profiles, have therapeutic potential, particularly in topical treatments for skin conditions and as ingredients in health-promoting products[21].

In line with international standards, such as the IWTO-10 analysis method, our research confirmed that wool oil extracted using ethanol met the recommended criteria for acid and saponification numbers, further validating the suitability of ethanol as an effective solvent for lanolin extraction. This method offers a significant advantage over traditional solvents like dichloromethane, which have raised health concerns due to their carcinogenic properties[22]. Given the increasing demand for natural and safe ingredients, the results from our study could pave the way for the development of sustainable, cost-effective methods for lanolin extraction in Mongolia[9].

In conclusion, this study highlights the potential of Mongolian sheep's wool as a valuable resource for extracting high-quality wool oil. By utilizing ethanol as the solvent in the Soxhlet method, we have established a more efficient and eco-friendlier alternative to conventional methods, supporting the development of local industries and contributing to the production of natural products with high commercial and medicinal value[23]. Future research should focus on optimizing the extraction process further, exploring additional applications of wool oil, and investigating the long-term stability and quality of the extracted lanolin[4]. Strengthening local lanolin extraction

and processing aligns with global trends towards sustainable, natural ingredients and can position Mongolia as a competitive player in the international lanolin market.

CONCLUSION

The optimal method for extracting wool oil from sheep wool using the Soxhlet apparatus involves a 3-hour extraction period with ethanol (99.6%) as the solvent. Additionally, freshly sheared wool produces a higher oil yield compared to wool that has been stored for a prolonged period. Infrared spectroscopy analysis of the wool oil revealed the presence of functional groups such as C=O, CH3, CH2, C-C, C-O, and O-H. Furthermore, the fatty acid composition of the wool oil, analyzed through gas chromatography, identified 14 distinct fatty acids, with saturated fatty acids comprising the largest proportion (66.6%). Polyunsaturated fatty acids accounted for 26.26%, while monounsaturated fatty acids made up 7.14%.

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