# Phytochemical Screening and Biological Activities of the Oil Extracted From the Seeds of *Eriobotrya japonica* Lindl. Grown In Pakistan

Anul Warha<sup>1</sup> Shereen<sup>1,4</sup>, , Sohail Hassan<sup>1</sup>, Darakhshan Masroor<sup>2</sup>, Khwaja Ali Hasan<sup>3</sup>, , Yusra khan<sup>2,4</sup>, Syeda Rafia Zahra Rizvi<sup>2,4</sup> Iffat Sultana<sup>1</sup>

# **Abstract**

Plant metabolites are sources of phytochemicals potentially used as therapies in treating inflammatory and metabolic diseases. Eriobotrya japonica Lindl. (loquat), the species that belongs to Rosaceae family is a popular fruit cultivated and used in traditional medicine, whereas the fruit is eaten, seeds are usually discarded even though they also contain a reservoir of bioactive compounds. This current study was principally designed to study the phytochemical composition of E. japonica seed oil isolated from fruits grown in Pakistan., oil was extracted by n- hexane maceration and studied using GC-MS, revealed 14 main chemicals, mostly made up of derivatives of saturated fatty acids and triglyceride esters. Glycerol tricaprylate (11.97%), lauric acid esters (9.39%), and methyl esters of palmitic and capric acid compounds with known bioactive properties were among the notable components. Albino Wistar rats were used for assessing biological activity. Using the paw edema method, anti-inflammatory activity was assessed, oil at 200 and 400 mg/kg indicated dose-dependent effects that were similar to those of ibuprofen (15 mg/kg). Oil was administered at 100, 200, and 400 mg/kg, and the anti-diabetic effect was assessed using random blood glucose levels and fasting during a 21-day period. Results demonstrated significant reductions in glucose levels relative to gibenclamide (5 mg/kg). Significant dose- and time-dependent responses were confirmed by statistical analyses employing Tukey HSD and Dunnett's t-test. This work finds that E. japonica seed oil have anti-inflammatory and antidiabetic activity due to the presence of a variety of phytochemicals.

Key words: Eriobotrya japonica Lindl. (loquat), GCMS, anti-diabetic and anti-inflammatory activity.

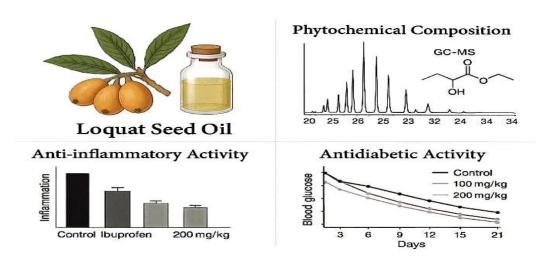
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<sup>&</sup>lt;sup>1</sup>Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi 75270, Pakistan

<sup>&</sup>lt;sup>2</sup>Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi 75270, Pakistan

<sup>&</sup>lt;sup>3</sup>Department of Biochemistry, University of Karachi, Karachi, 75280, Pakistan

<sup>&</sup>lt;sup>4</sup>Department of Pharmacy, Nazeer Hussain University, Karachi, Pakistan



Graphical abstract

# 1. Introduction

Medicinal plants have played a crucial role in the treatment of human beings since ancient times (Rabizadeh et al., 2022). Plants produce a wide range of phytochemicals compounds, which include flavonoids, alkaloids, terpenes, and polyphenols, which produce pharmacological properties such as free radical scavenging activity, analgesic, antimicrobic, hypoglycemic, and carcinogenic. (ayse and Jamwal et al. 2018, Sharma et al., 2018). The plant Eriobotrya japonica commonly known as loquat, belonging to the Rosaceae family, widely cultivated for its edible fruit and has a long-standing history in traditional medicine for treating conditions ranging from respiratory ailments to chronic diseases. Traditionally utilized in East Asian medicine, different parts of the Eriobotrya japonica plant, including leaves, fruits, seeds, and flowers, have been used to cure maladies such as bronchitis, cough, diabetes, and gastrointestinal issues. (Ibrahim al 2022).Loquat's fruit bioactive compounds include phenolic acids, carotenoids, terpenes, and flavonoids, all of which exhibit significant health benefits. These phytochemicals demonstrate anti-inflammatory, potent antioxidant, antidiabetic, and anticancer properties, alongside hepatoprotective hypolipidemic and effects(Amin et al 2025, husein et al 2018) Though E. japonica seed oil has been used for centuries and is becoming more and more popular in industry, little research has been done on its thorough phytochemical profiling. (Ahumda et al 2017). It has been discovered to be a group of several bioactive composites, such as triterpenoids, flavonoids, phenolics, and carotenoids, which shield the body from illness (Liu et al., 2016). Numerous in-vivo and in vitro biological investigations have revealed significant positive benefits linked to these bioactive substances. According to Narashans Alok Sagar et al. (2019), these effects include anti-inflammatory, anti-diabetic, anticancer, hepatoprotective, antioxidant, antiviral, and antiaging qualities. Therefore the current study was conducted to identified the phytochemicals of the Eriobotrya japonica Lindl. (loquat) seed oil and to evaluate which phytochemicals

responsible for the anti inflammatory and antidiabetic activity.

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## 2. METHODOLOGY

### 2.1 Collection and Extraction

Fresh fruits of *Eriobotrya japonica* Lindl. were gathered and identified from the University of Karachi's Botany Department. The pulp was extracted after the gathered fruits were cleaned with distilled water. After seven days, 500 g of shead dried seeds powder was macerated in n-hexane for a week. After the filtration oil was separated by using a rotary evaporator. (Xiao et al 2023,)

# 2.2Electron ionization Gas Chromatography— Mass Spectrometry (EI-GC-MS) Technique

The assessment of EI-GC-MS was conducted at the University of Karachi's H.E.J. Research Institute of Chemistry. In order to separate and identify the compounds present in the sample, a 0.5 μl sample was injected into a GC-System (Agilent Technologies 7890A.) that was coupled to a GC/MS Triple Quad Agilent Technologies 7000. The oven was first set to 50 °C for one minute, then 10 °C/min to 200 °C for five mins, and at last for 3°C/min to 310 °C for fifty mins. (Shaheed et al 2022, Kanjkar et al 2017)

# 2.3Acute Toxicity

The study was carried out in healthy albino mice. The randomly selected seven albino mice were—were stored to a suitable environment under the complete superintendence of laboratory premises for a week before the examination. The dosage was recommended based on the mice BMI values. Nonetheless, 200mg/kg and 400mg/kg were thought to be more efficient. The mice's survival rate was assessed after five days, and the activities were monitored for twenty-four hours. (Zhu et al 2022, Khan, Rajput, and others, 2017).

# 2.4 Anti-inflammatory activity

The paw edema method was widely used for the identification of anti-inflammatory activity. The 27 animals were randomized into 3 groups (A, B, and C) with 9 animals in each group, A is a control and normal saline was given to rats by oral route., B is a positive control standard group drug Ibuprofen dose 15mg/kg given orally, and C group received seed oil extracts of *Eriobotrya japonica* at the dose of 200mg/kg and 400mg/kg. A digital Plethysmometer was used to examine the animal's hind paw before to administering the medication. Acetic acid (2%), 0.05M, was administered subcutaneously to rats to induce an inflammation. Following 20 minutes of inflammation, the medication was administered . the size of the hind paw was measured hourly for continuous fiver hours.

# 2.5 Anti-diabetic activity:

(Angela et al 2020)

Healthy animals were given intraperitoneal injections of Alloxan monohydrate to induce diabetes. A single intraperitoneal injection of Alloxan monohydrate (120 mg/KgBW) diluted with normal saline based on each animal's BW was used to develop experimental diabetes in the mice, ensuring a glucose level of 150 mg/Kg. A blood glucose level was measured 72 hours after Alloxan monohydrate was consumed. The animals with diabetes were chosen when their blood glucose level was 250 mg/dl.The rats were examined at various intervals after being pierced in the tail vein to extract a tiny quantity of blood droplets. There were seven animals in each of the five groups (A, B, C,D and E) that were randomly selected from among the 35 animals. Both the diabetes control groups (Groups B, C,D and E) and the normal control group (Group A) were given normal saline every day. One diabetic group (Group B) was given glibenclamide 5 mg/kg, while three diabetic groups (Groups CD, and E) were given oil samples containing 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. For 21 days, the drugs were taken orally once every

day. A glucose meter was used to determine each animal's proper blood glucose level at regular intervals on the first, seventh, fourteen, and twenty-first, days.(Infanate et al 2024).

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# Ethical approval

On December 19, 2024, the study was conducted with ethical permission under ASRB/No./08103/Pharm

**2.6 Statistical analysis:** SPSS (version 22), was used to determine the mean result, standard error of mean, standard deviation, and ANOVA, and the post hoc Dunnet/Tukey test was used for multiple comparisons.

## 3. RESULT

# 3.1.Identification and Characterization of Chemical Compounds

The oil extracted from the seeds of *Eriobotrya japonica* Lindl. was subjected to gas chromatography (GC) and mass spectrometry (MS), which yielded detailed information about the oil's molecular makeup. All 14 compounds were identified and determined to be triglycerides or tri-esters with all three similar or dissimilar fatty acids (Figure 1, Table 1).

# 3.2 Fragmentation pattern of compounds The most important fragment ions found in the mass Spectrometry analysis are listed in Table 2, which is essential for determining the active compounds' structural identity.

To facilitate thorough structural elucidation, table 1 provides an exhaustive list of all fragment ions—major and minor—found in the mass spectroscopic study of the active compounds.

# 3.3Anti-inflammatory activity:

The study evaluated the anti-inflammatory effects of several drugs using the Rat PAW Edema technique. The treatments that were given were Ibuprofen (15 mg/kg), Normal Saline (10 ml/mg) as the control, and E. japonica at two doses (200 mg/mL and 400 mg/mL), as indicated in table 4. A comparison is made in Figure 2. Over the

course of the observation period, the extract (E. japonica 400 mg/kg) shows noticeably greater anti-inflammatory efficacy than ibuprofen (15 mg/kg). (Figure 3&4) with a peak activity more than twice as high as that of ibuprofen, the extract may have a stronger or more potent anti-inflammatory effect.

# 3.4 Anti-diabetic Effect

Table 5 summarizes the random blood glucose values that were measured at various dosages and intervals. This suggests a noticeable and significant

influence on glucose regulation. (Figure 5, Table 5) The different dosages shown varying degrees of decrease in fasting blood glucose levels over time. (See Figure 6 and Table 6) The study investigates the anti-diabetic effect of a drug by measuring fasting blood glucose levels in rats at different dosages (10 ml/kg, 100 mg/kg, 120 mg/kg, 200 ml/kg, and 400 ml/kg) and time points (0 Day, 7<sup>th</sup> Day, 14<sup>th</sup> Day, 21<sup>st</sup> Day). The anti-diabetic study suggest strong action with the dose of 400mg/kg as compared to other two doses.

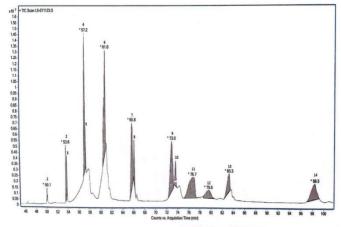


Figure 1: Characterization of Compounds by EI- Identification and Chemical GCMS analysis.

Table 1: Identified and unidentified compounds of the sample

 $C_{51}H_{98}O_{6}$ 

Table 2: Key fragmentation patterns of active compounds obtained from *Eriobotrya japonica* Lindl.

14

98.5

806

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Octadecanoic acid, 3-[1-(oxododecyl)oxy]-

1,2-propanediyl ester

<b>Com</b> p	Molecular	Key Fragments (m/z)	Identified as
	Peak (m/z)		
1	470	395, 327, 242, 201, 158, 127	Glycerol tricaprylate
2	554	536, 470, 383, 270, 229, 171, 155	Decanoic acid, 1,2,3-propanetriyl ester
3	638	536, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
4	638	564, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
5	806	592, 523, 298, 257, 183	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester
6	638	592, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
7	638	620, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
8	638	620, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
9	638	578, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
10	806	646, 523, 298, 257, 183	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester
11	806	649, 523, 298, 257, 183	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester
12	806	677, 523, 298, 257, 183	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester
13	806	620/621, 439, 298, 257, 183	Dodecanoic acid, 1,2,3-propanetriyl ester
14	806	634, 523, 298, 257, 183	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-

1,2-propanediyl ester

Table 3: Comparsion of anti-inflammatory response *Eriobotrya japonica* 200mg/kg and 400mg/kg with standard ibuprofen15mg/kg and control

Hour	Ibuprofen (15 mg/kg)	Normal Saline (10 ml/kg)	E.japonica (200 mg/kg)	E.japonica (400 mg/kg)
0	1.27 ± 0.01	1.28 ± 0.022	1.29 ± 0.026	1.28 ± 0.012
1	1.50 ± 0.031	2.32 ± 0.024	2.08 ± 0.019	1.98 ± 0.029
2	1.67 ± 0.036	4.19 ± 0.059	3.98 ± 0.038	3.63 ± 0.052
3	1.78 ± 0.027	5.12 ± 0.073	4.64 ± 0.042	3.83 ± 0.036
4	1.71 ± 0.035	5.28 ± 0.066	4.53 ± 0.072	3.63 ± 0.042
5	1.53 ± 0.027	5.08 ± 0.055	4.17 ± 0.072	3.36 ± 0.05

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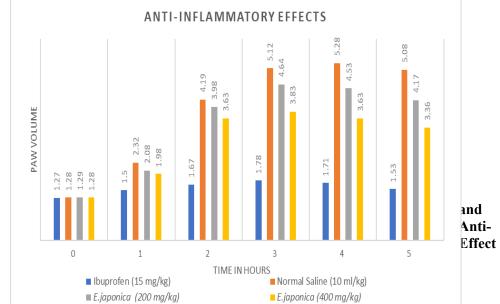


Figure 2: Analysis Interpretation of Inflammatory by Rat PAW Edema Method

Table 4: Anti -inflammatory Effect by Rat PAW Edema Method

AFTER DRUG POW SIZE								
	0hr	1hr	2hrs	3hrs	4hrs	5hrs		
	Befor				L			
EXTRACT &	е							
DOSES	Drug		Mean ± SEM					
DOSES	POW							
	size							
Ibuprofen	1.27±	1.50±	1.67±	1.78±	1.71±	1.53±		
15mg / kg	0.01	0.031	0.036	0.027	0.035	0.027		
Normal Saline	1.28±	2.32±	4.19±	5.12±	5.28±	5.08±		
10 ml / kg	0.022	0.024	0.059	0.073	0.066	0.055		
E.japonica	1.29±	2.08±	3.98±	4.64±	4.53±	4.17±		
200 mg /kg	0.026	0.019	0.038	0.042	0.072	0.072		
E.japonica	1.28±	1.98±	3.63±	3.83±	3.63±	3.36±		
400 mg / kg	0.012	0.029	0.052	0.036	0.042	0.05		

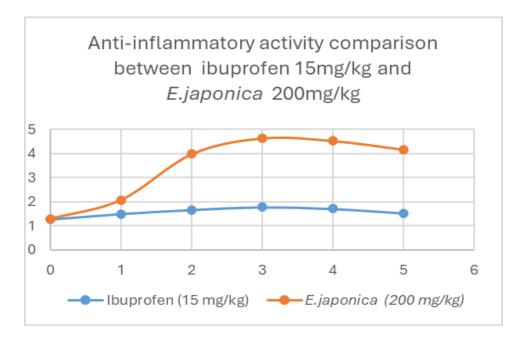


Figure 3: Comparison of Anti-inflammatory activity between Ibuprofen and E.japonica (200mg/kg)

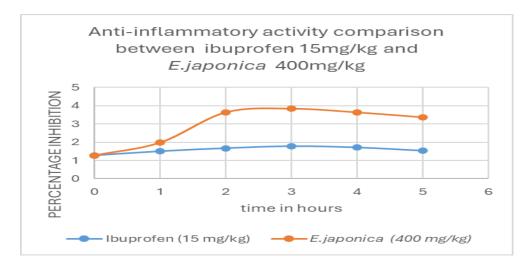


Figure 4: Comparison of Anti-inflammatory activity between Ibuprofen and E.japonica (400mg/kg)

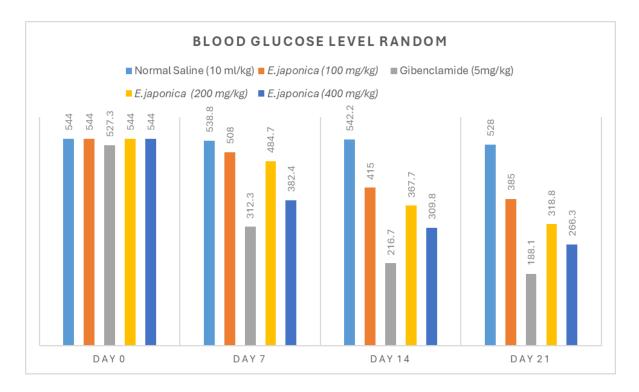
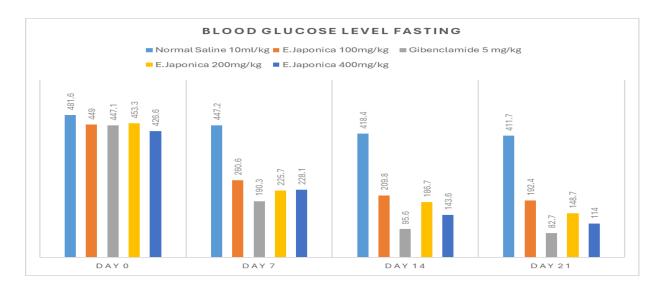


Figure 5: Random Blood Glucose Levels

Table 5: Blood Glucose level Random (Mean  $\pm$  SD)

Blood Glucose level Random (Mean ± SD)						
Day0 Day7 Day14 Day21						
Normal saline 10ml/kg	544 ± 0	538.8 ± 11.6	542.2 ± 2.7	528 ± 19.3		
Gibenclamide 5 mg/kg	527.3 ± 29.9	312.3 ± 22.2	216.7 ± 19.5	188.1 ± 21.4		
Eriobotrya japonica 100 mg/kg	544 ± 0	508 ± 17.7	415 ± 35.0	385 ± 14.6		
Eriobotrya japonica 200 mg/kg	544 ± 0	484.7 ± 13.1	367.7 ± 38.8	318.8 ± 35.0		
Eriobotrya japonica 400 mg/kg	544 ± 0	382.4 ± 43.3	309.8 ± 34.4	266.3 ± 19.0		



**Figure 6: Fasting Blood Glucose Levels** 

Table 6 Blood Glucose level Fasting (Mean  $\pm$  SD)

Blood Glucose level Fasting (Mean ± SD)							
TREATMENT Day0 Day7 Day14 Day2							
Normal Saline							
10ml/kg	481.6 ± 53.6	447.2 ± 80.8	418.4 ± 78.3	411.7 ± 89.6			
Gibenclamide							
5 mg/kg	447.1 ± 82.6	190.3 ± 23.3	95.6 ± 13.9	82.7 ± 14.6			
E. japonica							
100mg/kg	449 ± 72.8	260.6 ± 31.7	209.8 ± 28.9	192.4 ± 14.9			
E. japonica							
200 mg/kg	453.3 ± 70.2	225.7 ± 15.6	186.7 ± 12.6	148.7 ± 10.1			
E. japonica	100 0 1 01 5	222.4 . 22.2	440.6 : 47.4	44440.7			
400 mg/kg	426.6 ± 64.5	228.1 ± 28.9	143.6 ± 17.4	114 18.7			

# 5. Discussion

Medicinal plants are abundant sources of phytochemicals, which play essential roles in the treatment of inflammatory and metabolic disorders. The plant *Eriobotrya japonica* commonly known as loquat, belonging to the

Rosaceae family, widely cultivated for its edible fruit and has a long-standing history in traditional medicine for treating conditions

ranging from respiratory ailments to chronic disease *Eriobotrya japonic*a seeds have recently piqued the interest of numerous culinary and

medicinal firms due to their bioactive contents. (El-Marouani et al., 2020). Loquat seeds contain more antioxidants and polyphenols than the peel and flesh of the fruit. (Ammad et al., 2025, peng 2021), in traditional remedies (Gupta et al., 2020), and as a natural antioxidant. (Zhang et al., 2021).

Many food enterprises produce a lot of waste agricultural products, such seeds, which contribute to significant environmental issues and financial losses (Iqbal et al., 2021). Numerous studies on this topic have been carried out, and the findings suggest that the best sources of potentially useful bioactive compounds may be the plant waste components of food factories (Karimi et al., 2021; Patra et al., 2022; Ziemlewska et al., 2021). Most loquat seeds are consumed, however a significant portion are thrown away, squandered, and have little commercial value. In the realm of food science, natural ingredients are becoming more and more popular since they offer a variety of affordable bioactive components. Since synthetic chemicals are extremely harmful to human health, they are the best substitutes (Abid et al.,2024; Al-Zahrani et al., 2022; Ganesh et al., 2022).

In Pakistan, after the fruit flesh is consumed, the seeds are thrown away and never used again. (Rajalakshmi et al 2017). Thus, this study's goal was to examine the physiochemical traits and to examine the phytochemical composition and biological activities of seed oil extracted from Eriobotrya japonica Lindl. grown in Pakistan. Using Gas Chromatography-Mass Spectrometry (GC-MS), we identified 14 compounds, including triglycerides glycerol such as tricaprylate and dodecanoic acid esters, which are known for their bioactivity. These findings are consistent with our objectives, demonstrating the seed oil's medicinal potential and emphasizing its significance as a natural element in functional meals, nutritional supplements, cosmetics, and pharmaceuticals.

# 5.1 Analysis of Retention Indices and Confidence Intervals

The retention indices (RI) and confidence intervals for the discovered compounds provide valuable information about their chemical behavior and stability. The non-polar retention index values, calculated using an n-alkane scale, varied from 3143 iu to 5528 iu. These numbers represent the chemicals' respective retention periods during gas chromatography .For example, glycerol tricaprylate had a retention index of 3143 iu, indicating a lower retention period than Octadecanoic acid, 3-[(1oxododecyl)oxy]-1,2-propanediyl ester, which had a retention index of 5528 iu. The confidence ranges for these values, particularly the 50% (47 iu) and 95% (201 iu) intervals, provide insight into the precision and dependability of retention index estimates. This precision is required for the precise identification and quantification of chemicals in complicated mixtures. Some compounds have relatively similar retention times, such as compounds 2 and 3 with R.T. 53.6 min and 53.8 min, compounds 4 and 5 with R.T. 57.2 min and 57.3 min, and compounds 9 and 10 with R.T. 73 min and 73.7 min, respectively. Compounds with near R.T may be similar, hence the number of discovered compounds may be less than 14.

# **5.2** Comparative Analysis and Biological Implications

The discovered chemicals show that seed oil is high in medium- to long-chain triglycerides. These chemicals have important biological and nutritional implications. Long-chain triglycerides (LCTs), such as dodecanoic acid, 1,2,3-propanetriyl ester, and octadecanoic acid derivatives, are necessary for cellular structure and function. The presence of these triglycerides may contribute to the oil's possible health benefits, including as anti-inflammatory, antibacterial, and metabolic regulatory qualities. (Fiume et al 2023)

The GC-MS analysis produced 14 peaks, which were then interpreted to represent four distinct tri-esters. (Koch et al 2022, Mujtaba et al. 2020).Our investigation also identified Glycerol tricaprylate, Decanoic acid, 1,2,3-

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propanetriyl ester, Dodecanoic acid, 1,2,3-propanetriyl ester, and Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester. The identity was validated by comparing the fragmentation patterns to those found in the NIST library and literature references. Retention periods were measured for each chemical, and fragmentation patterns were examined to confirm the structures.

Further research into the biological actions of these chemicals could provide important insights into seed oil's medicinal and economic possibilities. Stearic acid (Octadecanoic acid, C18) is derived from Octadecanoic acid, 3-[(1oxododecyl)oxyl. The seed oil's saturated fatty (-1,2-propanediyl acids ester, 1.2.3propanetriyl ester, and caprylic acid (C8) from glycerol tricaprylate) are particularly resilient to oxidative stress due to their lack of double bonds. According to the study, oil extracted from loquat seeds can be utilized in medicinal and cosmetic products and stored safely for a long time. (Narshans et al 2019). Given the oil's high quantity and fatty acid composition, more research into its use in culinary and non-food products is recommended.

According to the study, there is a dosedependent anti-inflammatory activity of loquat oil. It reduces inflammation more effectively and sustainably at a greater dosage (400 mg/kg) than at a lower dosage (200 mg/kg). As a positive control, ibuprofen exhibits a potent anti-inflammatory effect. experimental setup is validated by the normal saline control, which verifies the anticipated inflammatory response. When the activity is compared to the sample and standard, the results show that the extract is highly effective in reducing inflammation, possibly more so than ibuprofen at the concentrations tested. The extract at 200 mg/kg shows a similar pattern of activity to the 400 mg/kg concentration, but the peak activity in the 400 mg/kg extract is slightly less than in the 200mg/kg. quantities of the oil showed higher activity than ibuprofen, suggesting that the Eriobotrya japonica oil is an effective anti-inflammatory medication. These findings suggest that *Eriobotrya japonic*a oil have therapeutic effects in the treatment of inflammation, or due to secondary active metabolites .

The ANOVA and Tukey HSD post hoc tests revealed that the varied treatment doses used on different days had a significant impact on glucose levels. For dosages more than 100 mg/kg, glucose levels gradually and noticeably decline over time. Higher dosages result in the biggest decreases (100 mg/kg, 200 mg/kg, and 400 mg/kg), with considerable drops in glucose levels by Days 14 and 21. The fact that the 100 mg/kg dose showed no apparent fluctuations over time suggests that this dose may not have a significant effect on blood glucose levels. The findings support the efficacy of higher dose regimens in reducing blood glucose levels by showing that higher dosages of the medicine had a greater effect on lowering glucose levels.

Significant anti-diabetic effects at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg. The ANOVA results indicate considerable anti-diabetic benefits. The impact is most obvious at higher doses, as fasting blood glucose levels steadily drop. The oil is a source of n-octadecanoic acid, and by increasing the concentration of an acid in the oil, the antidiabetic activity of the oil may increase.

### Conclusion

The current study indicated the significant pharmacological potential of Eriobotrya japonica oil, which has traditionally been underused despite the loquat plant's widespread therapeutic use. This study discovered *E. japonica* seed oil is a rich source of bioactive phytochemicals with potent anti-inflammatory and anti-diabetic effects. Its complex lipid composition likely contributes synergistically to these activities. These results validate traditional uses of the plant and point to new applications of its seed oil in natural health product development. With further research, this oil could emerge as a valuable addition to the arsenal of plant-based therapeutics for managing metabolic and inflammatory diseases.

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118-133

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