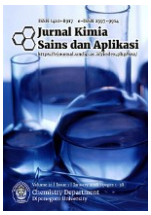
ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 28 (8) (2025): 442-451

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa



Chemical Composition, Physicochemical, and Pharmacokinetics Profile of Custard Apple Leaf (*Annona squamosa*) Essential Oil Extract

Khrisna Pangeran [1], Khairul Anam [2,*], Dewi Kusrini [3]

[1] Department of Pharmacy, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, Jakarta, Indonesia
[2] Department of Pharmacy, Faculty of Medicine, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, Semarang 50275, Indonesia
[3] Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof. Soedarto, SH, Tembalang, Semarang 50275, Indonesia

* Corresponding author: k.anam@live.undip.ac.id

https://doi.org/10.14710/jksa.28.8.442-451

Article Info

Article history:

Received: 04th August 2025
Revised: 05th October 2025
Accepted: 06th October 2025
Online: 31st October 2025

Keywords:
custard apple; steam distillation; volatile; Psychochemical; Pharmacokinetics

Abstract

This study investigates the effects of different drying methods on the chemical composition, physicochemical characteristics, and pharmacokinetic properties of custard apple (*Annona squamosa*) leaf essential oils. The research also evaluates how these drying techniques influence the yield and quality of the oils, with particular emphasis on terpenoid content and their pharmacological relevance. Fresh custard apple leaves were subjected to four drying methods: shade drying, sun drying, oven drying at 45°C, and air conditioning (AC) drying at 20°C. Essential oils were extracted through steam distillation and analyzed using GC– MS to determine their chemical constituents. Various physicochemical parameters, including refractive index, specific gravity, acid value, and saponification value, were measured. Pharmacokinetic properties were assessed using the pkCSM online tool, while network visualization was performed via STRING.db. The drying methods significantly affected the essential oil yield, with oven drying producing the highest yield. Based on physicochemical analysis, shade drying resulted in the highest acid and ester values. The chemical profiles varied across treatments, with trans-β-caryophyllene, germacrene D, and α- humulene identified as the dominant compounds in all samples. Network pharmacology analysis indicated that CYP2C9 and CYP2C19 play critical roles in optimizing personalized therapy by minimizing adverse effects and enhancing drug efficacy across various diseases.

1. Introduction

The utilization of natural ingredients as the foundation of the herbal-based chemical industry in Indonesia has recently increased. Several natural materials, including essential oils derived from various Indonesian herbal simplisia, have even been produced on a large scale. Custard apple (*Annona squamosa* L.) belongs to the family Annonaceae, which comprises approximately 2,300–2,500 species and more than 130 classified genera. This plant is distributed across Southeast Asia, including Malaysia, Indonesia, Cambodia, Laos, and Vietnam. Several species within the Annonaceae family possess leaves with a distinctive aroma due to the presence of volatile compounds, primarily terpenoids [1].

Two major classes of compounds that have been widely investigated for their pharmacological activities in custard apple plants are acetogenins and essential oils. In fresh custard apple leaves collected from western India, a total of 28 compounds were identified, with β-cedrene (23%) and β-caryophyllene (14%) being the predominant constituents. The decoction of custard apple leaves has traditionally been used to treat fever, cough, and digestive disorders, while crushed leaves are applied externally to treat minor wounds and scratches due to their antibacterial activity [2]. As most natural products

are seasonally available and cannot always be obtained fresh, the preservation and storage of fresh simplicia are crucial. Unprocessed simplicia are susceptible to microbial contamination, which may develop rapidly under high moisture conditions [3].

The quality and safety of plants containing essential oils, which serve as raw materials for pharmaceuticals, aromatherapy, and food additives, are of paramount importance from a consumer perspective. Several factors influence the volatility and stability of essential oils, including drying, distillation, harvesting procedures, and storage techniques. Among these, drying is a simple preservation method that can be performed either naturally or artificially. While drying enables long-term storage and mass preservation, it also leads to the loss of volatile constituents, including essential oils [4]. Volatile aroma compounds are the most sensitive components during the drying process. Numerous studies have investigated the effects of drying on the essential oil composition of various aromatic plants, fruits, and vegetables, revealing that changes in volatile compound concentrations depend on several parameters, such as the drying method and conditions (e.g., temperature, air velocity, and relative humidity) [5].

In a previous study, essential oils from custard apple leaves collected from the dried mountainous regions of India were isolated using steam–water distillation and analyzed by GC–MS. The essential oil of Annona squamosa was found to be dominated by sesquiterpene compounds (85.2%), with (E)-caryophyllene (15.9%), γ- cadinene (11.2%), epi-α-cadinol (9.4%), (Z)-caryophyllene (7.3%), γ-muurolene (5.4%), α-humulene (5.2%), and viridiflorene (5.0%) identified as the major constituents [6].

In another study, essential oils extracted from Myrtus communis leaves subjected to shade drying contained 1,8-cineole (also known as eucalyptol, 33.495%) and linalool (29.217%) as the predominant components, followed by linalyl anthranilate (9.048%) and α-terpineol (7.158%). The study concluded that the essential oil of *Myrtus communis* leaves represents a potential source of natural antioxidants and antibacterial agents, attributed to its physicochemical characteristics, including solubility, refractive index, optical rotation, specific rotation, density, specific gravity, acid value, saponification value, and ester value [5, 7].

The primary purpose of drying is to reduce the mass of plant materials by removing water and volatile compounds through evaporation [8, 9], thereby inhibiting enzymatic degradation and limiting microbial growth so that the product can be stably stored for extended periods. Previous studies have demonstrated that different drying methods influence the major components of essential oils and significantly affect the relative percentage of their constituents as analyzed by GC–MS [10, 11].

A study comparing four drying methods, shade drying, oven drying, microwave drying, and freeze drying, of lemon balm essential oil concluded that the use of an appropriate drying temperature does not necessarily reduce the quality or quantity of essential oil, but alters its chemical composition [12]. Furthermore, the storage of *Annona squamosa* leaves was reported not to reduce the overall efficacy of organic extracts in suppressing the emergence of adult *Vigna unguiculata* (cowpea) pests. Therefore, the present study aims to evaluate the effect of different drying methods on the chemical composition and physicochemical properties of custard apple leaf essential oils.

2. Experimental

2.1. Materials and Tools

Fresh custard apple (*Annona squamosa*) leaves, NaOH solutions (0.1 N and 0.2 N), distilled water, technical ether (60%), HCl (0.5 N), technical ethanol (96%), and 1% phenolphthalein indicator were used in this study.

The equipment utilized included standard laboratory glassware (Pyrex), trays or pans, an oven, an electric stove, a long-tube condenser, extraction funnels, a 25 mL burette, a 5 mL pycnometer, an Atago refractometer, a Mettler Toledo digital balance, weighing bottles, scissors, a water pump, hoses, adhesive tape, clamps, a stand (stative), a water bath, and a Gas Chromatography–Mass Spectrometry (GC–MS) instrument (Shimadzu QP2010-SE).

2.2. Sample Preparation

Fresh custard apple leaves were collected from the Tembalang area, Semarang, Central Java, with a total weight of 10 kg (2.5 kg allocated for each drying method). The leaves were cleaned to remove dust and debris, separated from the leaf veins, and chopped into smaller pieces to increase the surface area.

2.3. Drying Method

The drying process was carried out using four different methods: shade drying, sun drying, oven drying at 45°C, and air conditioning (AC) drying at 20°C. Each drying process was conducted for 6 hours per day over three consecutive days. The dry weight of the samples was determined to measure the percentage of mass loss due to the drying process and to calculate the moisture content and residual compounds retained after drying, based on the gravimetric method [13]. The percentage of drying shrinkage was calculated using Equation (1), while the moisture content was determined using Equation (2).

$$Drying\ shrinkage\ (\%) = \frac{(W_{fresh} - W_{dry})}{W_{fresh}} \times\ 100\% \qquad (1)$$

$$Moisture\ content\ (\%) = \frac{(W_{initial} - W_{final})}{(W_{initial} - W_{cup})} \times\ 100\% \qquad (2)$$

Where, W_{fresh} is the fresh sample weight (g), W_{dry} is the dried sample weight (g), $W_{initial}$ is the initial weight before drying (g), W_{final} is the final weight after drying (g), and W_{cup} is the weight of the empty cup (g).

2.4. Characterization of Essential Oil Isolates

The dried samples were vacuum-packed and subjected to steam distillation for 6 hours to obtain the distillate in the form of custard apple leaf essential oil. The resulting isolates were analyzed organoleptically to

evaluate the color and aroma of the oil. The volume and yield were measured based on both fresh and dry sample weights. The density and mass of the oil were determined using a 5 mL pycnometer [14]. The yield of essential oil was calculated using Equation (3).

$$Yield\ (\%) = \frac{Total\ oil\ (g)}{Sample\ mass\ (g)} \times\ 100\% \quad (3)$$

2.5. GC-MS Conduction Analysis

The essential oil isolates obtained from each drying method were analyzed using a GC–MS instrument. A 0.10 µL sample of each essential oil was injected for analysis. The separation was performed on a non-polar Rtx-5MS capillary column (30 m length, 0.25 µm film thickness, and 0.25 mm internal diameter). The column temperature was programmed from 60°C to 200°C, with a carrier gas pressure of 12.7 kPa and a total flow rate of 44.8 mL/min.

Compound identification was conducted by interpreting chromatograms and mass spectra based on the mass-to-charge ratio (m/z) of the detected ions. The resulting spectra were compared with reference data to determine the chemical composition of the essential oils [15].

2.6. Physicochemical Test

Essential oils are complex mixtures composed of various constituents, including hydrocarbons, alcohols, acids, ethers, esters, aldehydes, and ketones. Their chemical quality can be assessed by determining parameters such as acid, ester, and saponification values. Meanwhile, their physical properties can be evaluated based on refractive index, specific gravity, yield, and organoleptic characteristics, including color and aroma [16].

2.7. Refractive Index Test

The refractive index of an essential oil is defined as the ratio of the speed of light in air to the speed of light in the oil at a specified temperature. This parameter is closely related to the chemical composition of the essential oil. The refractive index was measured using an Atago refractometer at a controlled temperature of 25°C. Observations were recorded when the bright and dark boundary lines coincided at the center of the cross-shaped scale on the instrument lens [17].

2.8. Specific Gravity Test

The specific gravity of an essential oil is defined as the ratio between the mass of the oil and the mass of an equal volume of water at the same temperature. This parameter is often correlated with the mass fraction of the components present in the essential oil. Measurements were conducted using a 5 mL pycnometer [18]. The specific gravity was calculated using Equation (4).

$$d = \frac{m_2 - m}{m_1 - m} \quad (4)$$

Where, m_2 is the mass of the object when submerged in the fluid (g), m_1 is the mass of the object in air (g), and m is the mass of the displaced fluid (g).

2.9. Acid Number Test

The acid number is expressed as the number of milligrams of 0.1 N NaOH required to neutralize the free fatty acids in 1 gram of oil. The endpoint is reached when the solution changes color from clear to pink and remains stable for at least 30 seconds. For the analysis, approximately 1 gram of essential oil was weighed into an Erlenmeyer flask, followed by the addition of 5 mL of 96% ethanol and three drops of 1% phenolphthalein indicator. The solution was titrated with a standard 0.1 N NaOH solution until the first appearance of a pink color that persisted for at least 10 seconds, indicating the endpoint of the titration. The acid number was calculated using Equation (5) [19].

$$\text{Acid number} = \frac{V \times N \times MW_{NaOH}}{m} \quad (5)$$

Where, V is the titration volume (mL), N is the normality of NaOH (eq/L), MW_{NaOH} is the molecular weight of NaOH (g/mol), and m is the sample mass (g).

2.10. Saponification Value and Ester Value

Ester compounds play a crucial role in determining the characteristic aroma of essential oils. The determination of the ester number in essential oils is based on the saponification of esters using a standard alkaline solution, followed by back-titration of the excess alkali until the color of the solution changes. An amount of 1.5 g of the essential oil was weighed into an Erlenmeyer flask. Then, 5 mL of 96% ethanol and three drops of phenolphthalein indicator were added. The free acids were neutralized with 0.1 N NaOH solution (approximately five drops). Subsequently, 10 mL of 0.5 N alcoholic NaOH solution was added. A condenser was attached to the flask, and the mixture was heated in a water bath for an hour [20].

After heating, the flask was removed and allowed to cool to room temperature for 15 minutes. Then, three drops of phenolphthalein indicator were added, and the remaining alkali was titrated with 0.5 N HCl until the endpoint was reached. A blank titration was performed using distilled water in place of the oil sample. The saponification and ester values were calculated based on the difference in the volume of titrant required between the blank and the sample, as expressed in Equations (6) and (7).

$$Saponification\ value = \frac{(V_{blank} - V_{sample}) \times N_{HCl} \times MW_{NaOH}}{m} \quad (6)$$

$$Ester\ value = Saponification\ value\ -\ Acid\ number \quad (7)$$

Where, V is the volume (mL), N is the normality (eq/L), MW is the molecular weight (g/mol), and m is the sample mass (g).

2.11. Pharmacokinetic Analysis

Pharmacokinetic analysis was performed using pkCSM (Pharmacokinetics and Chemoinformatics Software Modeling), a computational tool designed to predict the pharmacokinetic behavior of chemical compounds. The pkCSM platform was employed to evaluate key pharmacokinetic parameters, including absorption, distribution, metabolism, and excretion

(ADME), as well as potential interactions between the compounds and biological proteins [21, 22].

3. Results and Discussion

3.1. Drying of Custard Apple Leaves

The leaves of aromatic plants are commonly dried prior to extraction to reduce their moisture content [7]. Drying also aims to produce simplisia that is less susceptible to microbial growth and physical degradation, thereby extending its shelf life and preserving the quality of the essential oils. However, the effect of a particular drying method on the retention or loss of volatile compounds is unpredictable and depends on both the chemical nature of the compounds and the characteristics of the plant material [7]. Data from the drying process indicated that the percentage of drying shrinkage is influenced by temperature and humidity, leading to a reduction in sample mass due to the evaporation of water and the loss of volatile constituents during the drying process.

Table 1 shows that oven drying at 45°C resulted in the highest drying shrinkage value based on fresh weight (a), while shade drying exhibited the highest drying shrinkage value based on dry weight (b). However, the shade drying process tends to proceed more slowly, which may influence the quality of the plant material. Prolonged drying time can lead to undesirable effects, such as color changes, degradation of bioactive compounds, or loss of nutritional value [23].

3.2. Characteristics of Essential Oil Isolates

Using the same distillation duration of six hours resulted in variations in the volume and percentage yield of essential oils obtained from each drying method. Among them, the oven drying method at 45°C produced the highest essential oil yield. This is likely because the controlled temperature and low humidity during oven drying ensured consistent moisture removal, unlike shade and sun drying, where temperature and humidity fluctuate depending on environmental conditions such as weather and season. The essential oils obtained from custard apple leaves across all drying methods appeared yellow to pale yellow in color and emitted a strong, characteristic aroma [9].

3.3. Characteristics of Essential Oils

Color and scent are descriptive parameters and are not considered indicators of material purity. The density values presented in Table 2 ranged from 0.720 to 0.736 g/mL, which are lower than that of water. Consequently, the essential oil isolates were clearly separated from the aqueous layer, appearing above the water phase in the extract. The oil mass was determined from the difference between the weight of the pycnometer filled with oil and that of the empty pycnometer. The mass of the oil was directly proportional to the density of the essential oils, with the wind-dried sample exhibiting the highest value [24].

3.4. Physicochemical Profile

The evaluation of physicochemical characteristics provides practical insights into the quality, suitability, and potential applications of plant-derived essential oils in daily use. The primary objective of this analysis is to assess the quality of the essential oils both qualitatively and quantitatively. Chemically, quality can be determined through parameters such as acid value, ester value, and solubility in alcohol, while physically, it can be assessed through refractive index, specific gravity, yield, and color observations [25, 26].

Table 1. Results of the drying process of custard apple leaves

Drying method	Fresh weight (g)	Dry weight (g)	%Drying shrinkage (a)	%Drying shrinkage (b)
Shade drying	2,500	804.95	67.802	14.815
AC temperature (20°C)	2,500	750.7	69.972	10.494
Sun drying	2,500	768.55	69.258	9.360
Oven temperature (45°C)	2,500	662.45	73.502	11.585

Note: %Drying shrinkage (a) refers to the percentage of weight loss during the drying process, while % Drying shrinkage (b) refers to the value obtained using the gravimetric method.

Table 2. Characteristics of essential oil isolates

Drying method	Color	Scent	Volume (mL)	Total mass (g)	%Yield (A)	%Yield (B)	Density (g/mL)
Shade drying	Yellow	Quite strong	5.6	4.122	0.165	0.512	0.736
AC temperature (20°C)	Pale yellow	Quite strong	5.8	4.182	0.167	0.557	0.721
Sun drying	Yellow	Quite strong	5.6	4.032	0.161	0.525	0.720
Oven temperature (45°C)	Pale yellow	Quite strong	5.8	4.205	0.168	0.635	0.725

Note: %Yield (A) is calculated based on dry weight, and %Yield (B) is calculated based on wet weight.

Table 3. Physicochemical profile data of all drying methods

Drying method	Specific gravity	Refractive index	Acid value	Saponification value	Ester value
Shade drying	0.740	1.487	2.799	32.664	29.864
AC temperature (20°C)	0.724	1.423	2.399	19.999	17.599
Sun drying	0.725	1.474	1.399	8.666	7.266
Oven temperature (45°C)	0.729	1.442	1.399	12.666	11.266

3.5. Refractive Index

The refractive index is an important parameter for assessing the quality and purity of essential oils. In this study, the refractive index values ranged from 1.423 to 1.487. Variations in these values are influenced by the density of the medium and the concentration of terpenoids, as higher terpenoid content generally increases the refractive index. Among all drying methods, sun drying produced the highest refractive index value, indicating that the essential oil contained a higher proportion of volatile compounds with greater molecular density. The refractive index thus serves as a critical indicator of the chemical complexity of essential oils, reflecting the presence of dense compounds such as terpenoids. Higher refractive index values often correspond to more chemically complex oils, which can influence their therapeutic efficacy. Therefore, variations in refractive index across different drying methods may provide valuable insights into how each method affects the chemical profile and overall quality of the oil [27].

3.6. Specific Gravity

The specific gravity values of the essential oils ranged from 0.724 to 0.740, influenced by the mass fraction of their constituent compounds. A higher mass fraction of components such as sesquiterpenes, monoterpenes, and hydrocarbon chains generally increases the specific gravity of essential oils. The shade-dried sample exhibited the highest specific gravity value, likely due to the presence of long-chain molecules and a higher number of unsaturated bonds or oxygen-containing groups formed through oxidation reactions [28].

3.7. Acid Number

The acid value is an indirect measure used to determine the amount of free fatty acids in oil samples and to assess their edibility. The acid values of the essential oils ranged from 1.399 to 2.799. Higher acid values can negatively affect the quality of essential oils and alter their characteristic aroma. This increase may result from prolonged storage or exposure of the oils to light and air, leading to oxidation within storage containers. The shade-dried sample exhibited a higher acid value, likely due to its elevated free fatty acid content, which increases the susceptibility of the oil to oxidation upon contact with light and air [29].

3.8. Saponification Value and Ester Value

The saponification value was obtained in the range of 8.666–32.664, with the shade drying method showing the highest value. The saponification value influences the ester value, as it results from the reaction between free fatty acids and excess alkali. The ester values ranged from 7.266–29.864, with shade drying also producing the highest value. A higher ester value indicates better essential oil quality, as it contributes to a more distinctive and long-lasting aroma depending on the storage conditions. Prolonged distillation time may further increase the ester value due to a higher yield of essential oil obtained [30].

The differences observed in Table 3 among the drying methods are attributed to variations in the chemical composition of the essential oils resulting from each drying treatment. The physicochemical profiles of custard apple leaf essential oils can be compared across the different drying methods; however, they cannot yet be compared with official standards or published references, as there is currently no established standardization for custard apple leaf essential oil [31]. Overall, the shade drying method exhibited the highest average physicochemical values among all methods, whereas the oven drying method at 45°C produced essential oil isolates with the most stable characteristics.

3.9. Identification of Essential Oils Using GC-MS

A total of 37 compounds were identified across all drying methods, as presented in Table 4. The dominant group of compounds in the essential oil isolates consisted of hydrocarbons, both long-chain and short-chain, cyclic and functionalized, corresponding to compounds numbered 1–25, which accounted for 69.44% of the total composition. The remaining compounds belonged to the terpenoid group, including monoterpenes (C_{10}) and sesquiterpenes (C_{15}), represented by compounds numbered 25–36, contributing 30.56% of the total composition.

Peak identification was carried out by comparing the mass spectra of the detected peaks with those in the WILEY7.LIB database. Several compounds were identified across all drying methods, with the most prominent peaks corresponding to 3-methyl-2-pentanone (1), trans-β-caryophyllene (27), α-humulene (28), germacrene-D (29), and germacrene-B (30). Some characteristic compounds appeared exclusively in specific drying methods; for instance, camphene (1.48%) and α-pinene (1.05%) were detected only in shade drying, while δ-cadinene (2.51%) was unique to sun drying.

Table 4. Data on the components of the custard apple leaves' essential oil

No	Chemical component	%Area value			
		Sun drying	Shade drying	AC temperature at 20°C	Oven temperature at 45°C
1	3-methyl-2-pentanone	3.74	2.86	5.14	6.14
2	2-methyl-heptane	1.45	1.37	1.97	2.26
3	methyl-benzene	2.47	2.04	3.15	3.65
4	cis-1,3-dimethyl-cyclohexane	-	1.07	1.41	1.83
5	Octane	1.73	1.92	2.31	3.33
6	ethyl-cyclohexane	-	-	0.33	-
7	2-methyl-1,1-diethoxy-propane	-	1.34	1.56	1.75
8	2-methyl-octane	-	-	1.29	1.38
9	3,5-dimethyl-heptane	-	-	1.49	1.84
10	1,3-dimethyl-benzene	1.66	1.28	2.2	2.38
11	Nonane	2.78	1.84	3.04	1.84
12	3-methyl-nonane	-	-	0.96	-
13	propyl-cyclohexane	-	-	1.47	1.37
14	iso-amyl-phenyl-acetate	-	-	1.86	1.7
15	Decane	2.5	2.01	4	3.17
16	3,3-dimetil-octane	-	-	1.26	-
17	1-chlorohexadecane	-	-	1.29	-
18	trans-2-undecane-1-ol	-	-	1.43	-
19	Undecane	2.57	1.91	4.32	3.4
20	Dodecane	1.6	1.09	2.74	2.12
21	2,6-dimetil-undecane	-	-	1.27	-
22	2,3,7- trimethyl-octane	-	-	1.46	-
23	Heptadecane	-	-	1.66	-
24	Heptane	1.3	-	-	-
25	1-chlorodecane	3.61	-	-	-
26	cis- caryophyllene	9.14	7.16	5.4	-
27	iso-caryophyllene	7.11	-	-	6.9
28	trans-β-caryophyllene	33.74	33.64	22.06	29.98
29	α-humulene	5.61	5.48	4.58	4.47
30	Germacrene-D	8.97	12.83	7.69	9.84
31	Germacrene-B	4.63	7.01	4.7	5.49
32	δ-cadinene	1.36	1.77	1.56	-
33	δ-elemene	1.51	2.96	1.64	2.57
34	(-)-β-elemene	-	7.89	4.77	-
35	α-pinene	-	1.05	-	-
36	Camphene	-	1.48	-	-
37	γ-cadinene	2.51	-	-	-
	Total	99.99	99.99	99.99	99.99

Table 5. List of unique ("typical") compounds identified in essential oils from each drying method

Shade drying	AC temperature (20°C)	Sun drying	Oven temperature (45°C)
α-pinene	ethyl-cyclohexane	heptane	2-methyl-octane
camphene	3-methl-nonane	1-choro-decana	prophyl-cyclohexane
(-)-β-elemene	3,3-dimethyl-octane	γ-cadinene	iso-amyl-phenyl-acetate
-	1-chlorohexadecane	iso-caryophyllene	iso-caryophyllene
-	2,6-dimethyl-undecane	-	3,5-dimethyl-heptane
-	2,3,7-trimethyl octane	-	-
-	Heptadecana (-)-β-elemene	-	-

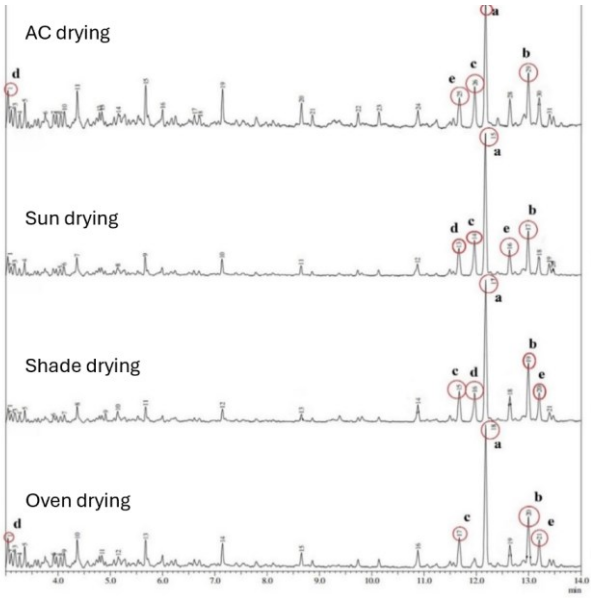


Figure 1. GC chromatogram overlay of essential oil isolates obtained from different drying methods

Figure 1 illustrates that peaks A and B represent two major compounds that appeared in nearly all drying methods but at different retention times, resulting in variations in their relative % area. Trans-β-caryophyllene, represented by peak A, was detected in all drying methods, exhibiting the highest relative abundance under sun drying (33.74%). Peak B, corresponding to germacrene-D, showed the greatest abundance in the shade drying method, with a % area of 12.83%. Germacrene-B and (−)-β-elemene were also most abundant in the shade drying method, with % areas of 7.01% and 7.89%, respectively. Meanwhile, 3-methyl-2-pentanone exhibited its highest concentration in the oven-drying method at 45 °C, with a % area of 6.14%.

Different drying methods yield distinct compound profiles, with variations in both intensity and presence of specific components. Drying under AC temperature at 20°C produced the greatest diversity of identified compounds, totaling 31 compounds. Sun drying resulted in 20 identified compounds, shade drying in 21 compounds, and oven drying at 45°C in 21 compounds. This suggests that each drying method generates a unique chemical profile characterized by specific compounds, even though all samples originated from the same raw material (Table 5).

Figure 2 shows that the essential oil isolated from sun-dried samples exhibited the highest intensity of trans-β-caryophyllene (33.74%) and α-humulene (5.61%), while the proportion of Germacrene-B (4.63%) was lower compared to other methods. Shade drying produced the highest intensity of Germacrene-D (12.83%) but had the lowest content of 3-methyl-2-pentanone (2.86%). In contrast, trans-β-caryophyllene (22.60%) appeared at a lower percentage in AC drying at 20°C. The oven drying method at 45°C yielded a smaller proportion of α-humulene (4.47%) but showed a higher content of 3-methyl-2-pentanone (6.14%) compared to the other methods [32, 33].

3.10. Physicochemical and Pharmacokinetic Properties

Based on Table 6, LogP is a parameter that indicates the solubility of a compound in water versus fat, reflecting its degree of hydrophobicity. The LogP values of all compounds listed are above 4, with trans-β-caryophyllene, germacrene-B, and α-humulene showing values between 4.73 and 5.03, suggesting strong lipophilic characteristics. In contrast, 3-methyl-2-pentanone exhibits a lower LogP value (1.62), indicating higher hydrophilicity [26].

Table 6. Analysis of pharmacokinetics and physicochemical properties using pkCSM online

Compound	Mol weight (g/mol)	Log P	H-donor	H-acceptor	Inhibitor (Protein)	Toxicity (LD_{50})	Bio score
Trans-β-caryophyllene	204.35	4.73	0	0	CYP2C19; CYP2C9	1.62	0.55
Germacrene-B	204.35	4.73	0	0	CYP2C9	1.73	0.55
α-humulene	204.35	5.03	0	0	CYP2C9	1.76	0.55
3-methyl-2-pentanone	100.16	1.62	0	1	CYP2C9	1.882	0.55
Germacrene-D	204.35	4.89	0	0	CYP2C9	1.63	0.55

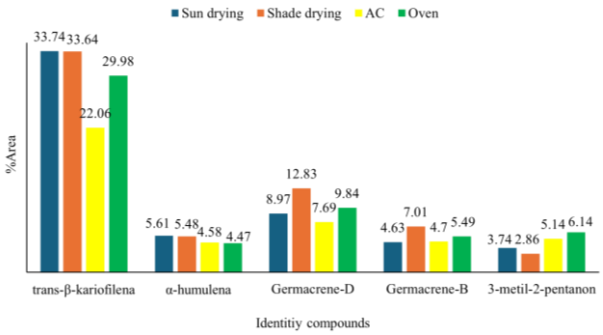


Figure 2. Diagram of the dominance of identified compounds in essential oils obtained from all drying methods

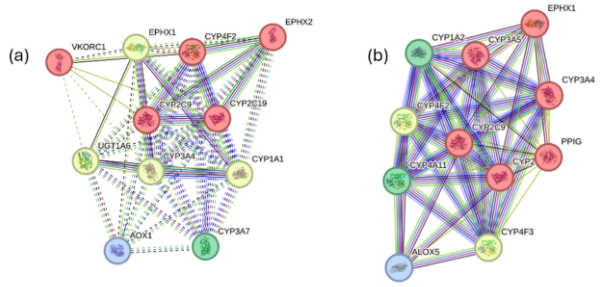


Figure 3. Network and interaction patterns between cytochrome P450 enzymes: (a) CYP2C9 and (b) CYP2C19

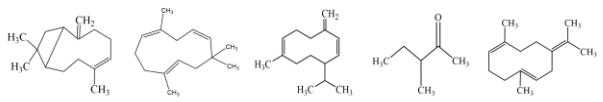


Figure 4. Most abundant identified compounds in custard apple leaf essential oil

Compounds with high LogP values tend to penetrate cell membranes more easily and may accumulate in fatty tissues, although they generally exhibit limited water solubility. All identified compounds show relatively low LD_{50} values, suggesting a moderate potential for toxicity. Among them, 3-methyl-2-pentanone has the highest LD_{50} value (1.882), indicating lower toxicity compared to the others, whose LD_{50} values range from 1.62 to 1.76. A lower LD_{50} value corresponds to a higher risk of adverse effects under excessive or uncontrolled exposure [6].

These findings emphasize the importance of understanding the relationship between physicochemical properties and pharmacokinetics in drug development, particularly for predicting absorption, distribution, metabolism, and excretion (ADME) profiles. The identified compounds were also analyzed using STRING.db to explore potential metabolic interactions with cytochrome P450 enzymes (CYP2C9 and CYP2C19) [22, 25]. Figure 3 presents two network diagrams illustrating the interactions and relationships between various cytochrome P450 (CYP) enzymes and other metabolic factors.

The compounds shown in Figure 4 are primarily metabolized by CYP2C9 and CYP2C19, which are key enzymes involved in drug metabolism [34]. Genetic polymorphisms or drug interactions that alter the activity of these enzymes can markedly influence the pharmacokinetic profiles of the compounds, particularly in the treatment of diseases such as rheumatoid arthritis, hypertension, breast cancer, Alzheimer's disease, and chronic pain. These findings highlight the importance of considering CYP2C9 and CYP2C19 in the design of therapeutic strategies and the personalization of treatment plans to minimize adverse effects and optimize drug efficacy [35, 36].

4. Conclusion

The findings of this study demonstrate that different drying methods significantly affect the physicochemical properties, yield percentage, drying shrinkage, and chemical composition of custard apple leaf essential oils. The most consistently identified compounds were trans-β-caryophyllene, α-humulene, germacrene D, germacrene B, and 3-methyl-2-pentanone. The shade-drying method produced higher physicochemical values and a greater concentration of the dominant compound, trans-β-caryophyllene, whereas drying at an air-conditioned temperature of 20°C resulted in the greatest diversity of identified chemical constituents compared to other methods.

References

[1] Lashin S. Ali, Gamal Mohamed, Ahmed A. El-Mansy, Khaled S. El-Bayoumi, Mohammed Y. Sobhey, Abd El-Fattah B. M. El-Beltagy, Reham A. Ghanem, *Annona squamosa* (custard apple) leaves extract mitigates abemaciclib-induced liver injury (AILI) in male rats, *Arab Journal of Basic and Applied Sciences*, 32, 1, (2025), 150-164 https://doi.org/10.1080/25765299.2025.2516880

[2] Jeevan Ankush Gawade, G. S. Gore, R. R. Bendgude, A comprehensive review: exploring the anti-arthritic anti-inflammatory potential of *Annona squamosa* (custard apple) leaves, *International Journal of Therapeutic Innovation*, 3, 1, (2025), 3048-4626 https://doi.org/10.55522/ijti.v3i1.0062

[3] Rania F. Abou-El-magd, Olfat M. W. A. Elghareeb, Hanem A. E. El-sherbiny, Najamul Nisa, *Annona squamosa* (custard apple) fruit extract alleviates the oxidative stress and inflammation induced by doxorubicin in the liver of male rats, *Journal of Medical and Life Science*, 7, 1, (2025), 8-21 https://doi.org/10.21608/jmals.2025.405552

[4] Thi Thu Trang Nguyen, Thi Van Anh Le, Nhu Ngoc Dang, Dan Chi Nguyen, Phu Thuong Nhan Nguyen, Thanh Truc Tran, Quang Vinh Nguyen, Long Giang Bach, Dung Thuy Nguyen Pham, Microencapsulation of Essential Oils by Spray-Drying and Influencing Factors, *Journal of Food Quality*, 2021, 1, (2021), 5525879 https://doi.org/10.1155/2021/5525879

[5] Grant Thamkaew, Ingegerd Sjöholm, Federico Gómez Galindo, A review of drying methods for improving the quality of dried herbs, *Critical Reviews in Food Science and Nutrition*, 61, 11, (2021), 1763-1786 https://doi.org/10.1080/10408398.2020.1765309

[6] Gera Narendra, Shalki Choudhary, Baddipadige Raju, Himanshu Verma, Om Silakari, Role of Genetic Polymorphisms in Drug-Metabolizing Enzyme-Mediated Toxicity and Pharmacokinetic Resistance

to Anti-Cancer Agents: A Review on the Pharmacogenomics Aspect, *Clinical Pharmacokinetics*, 61, 11, (2022), 1495-1517 https://doi.org/10.1007/s40262-022-01174-7

[7] Saeid Hazrati, Kazem Lotfi, Mostafa Govahi, Mohammad-Taghi Ebadi, A comparative study: Influence of various drying methods on essential oil components and biological properties of *Stachys lavandulifolia*, *Food Science & Nutrition*, 9, 5, (2021), 2612-2619 https://doi.org/10.1002/fsn3.2218

[8] Jelena Bajac, Branislava Nikolovski, Ivana Lončarević, Jovana Petrović, Branimir Bajac, Saša Đurović, Lidija Petrović, Microencapsulation of juniper berry essential oil (*Juniperus communis* L.) by spray drying: microcapsule characterization and release kinetics of the oil, *Food Hydrocolloids*, 125, (2022), 107430 https://doi.org/10.1016/j.foodhyd.2021.107430

[9] Fotini Plati, Adamantini Paraskevopoulou, Micro- and Nano-encapsulation as Tools for Essential Oils Advantages' Exploitation in Food Applications: the Case of Oregano Essential Oil, *Food and Bioprocess Technology*, 15, 5, (2022), 949-977 https://doi.org/10.1007/s11947-021-02746-4

[10] Alicja Napiórkowska, Marcin Kurek, Coacervation as a Novel Method of Microencapsulation of Essential Oils—A Review, *Molecules*, 27, 16, (2022), 5142 https://doi.org/10.3390/molecules27165142

[11] Olga Obrezanova, Anton Martinsson, Tom Whitehead, Samar Mahmoud, Andreas Bender, Filip Miljković, Piotr Grabowski, Ben Irwin, Ioana Oprisiu, Gareth Conduit, Matthew Segall, Graham F. Smith, Beth Williamson, Susanne Winiwarter, Nigel Greene, Prediction of In Vivo Pharmacokinetic Parameters and Time–Exposure Curves in Rats Using Machine Learning from the Chemical Structure, *Molecular Pharmaceutics*, 19, 5, (2022), 1488-1504 https://doi.org/10.1021/acs.molpharmaceut.2c00027

[12] Felix Funk, Beat Flühmann, Amy E. Barton, Criticality of Surface Characteristics of Intravenous Iron–Carbohydrate Nanoparticle Complexes: Implications for Pharmacokinetics and Pharmacodynamics, *International Journal of Molecular Sciences*, 23, 4, (2022), 2140 https://doi.org/10.3390/ijms23042140

[13] Mohammad Moghaddam, Leila Mehdizadeh, Chapter 13 - Chemistry of Essential Oils and Factors Influencing Their Constituents, in: A.M. Grumezescu, A.M. Holban (Eds.) *Soft Chemistry and Food Fermentation*, Academic Press, 2017, https://doi.org/10.1016/B978-0-12-811412-4.00013-8

[14] I. T. S. S. Silva, M. J. B. Fernandes, R. A. Oliveira, L. D. Carvalho, P. A. Cortez, A. R. São José, A. O. Conceição, *Annona squamosa* L. (Annonaceae): Chemical bioprospection and biological activity in two phenological stages, *Applied Ecology and Environmental Research*, 14, 4, (2016), 133-147 https://doi.org/http://dx.doi.org/10.15666/aeer/1404_133147

[15] Alexandros Ch Stratakos, Anastasios Koidis, Chapter 4 - Methods for Extracting Essential Oils, in: V.R. Preedy (Ed.) *Essential Oils in Food Preservation, Flavor and Safety*, Academic Press, San Diego, 2016, https://doi.org/10.1016/B978-0-12-416641-7.00004-3

[16] Mijat Božović, Alberto Navarra, Stefania Garzoli, Federico Pepi, Rino Ragno, Esential oils extraction: a 24-hour steam distillation systematic methodology, *Natural Product Research*, 31, 20, (2017), 2387-2396 https://doi.org/10.1080/14786419.2017.1309534

[17] Jana Orsavova, Ladislava Misurcova, Jarmila Vavra Ambrozova, Robert Vicha, Jiri Mlcek, Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids, *International Journal of Molecular Sciences*, 16, 6, (2015), 12871-12890 https://doi.org/10.3390/ijms160612871

[18] Nibedita Jena, Gajender Tripathi, Sanjeet Kumar, *Plants & Secondary Metabolites, Volume V*, Ambika Prasad Research Foundation, India, 2025,

[19] Ravi Pratap Singh, Ashok Kumar Pattnaik, Mithun Rudrapal, Soumya Bhattacharya, *Annona squamosa* Linn.: A Review of Its Ethnobotany, Pharmacology, Phytochemistry, Toxicity, and Conservation Needs, *Pharmacognosy Magazine*, 21, 2, (2025), 403-412 https://doi.org/10.1177/09731296241281422

[20] Hesham H. A. Rassem, Abdurahman H. Nour, Rosli M. Yunus, Techniques for extraction of essential oils from plants: a review, *Australian Journal of Basic and Applied Sciences*, 10, 16, (2016), 117-127

[21] Alina A. Bagdasaryan, Vladimir N. Chubarev, Elena A. Smolyarchuk, Vladimir N. Drozdov, Ivan I. Krasnyuk, Junqi Liu, Ruitai Fan, Edmund Tse, Evgenia V. Shikh, Olga A. Sukocheva, Pharmacogenetics of Drug Metabolism: The Role of Gene Polymorphism in the Regulation of Doxorubicin Safety and Efficacy, *Cancers*, 14, 21, (2022), 5436 https://doi.org/10.3390/cancers14215436

[22] Maria Carolina Stipp, Alexandra Acco, Involvement of cytochrome P450 enzymes in inflammation and cancer: a review, *Cancer Chemotherapy and Pharmacology*, 87, 3, (2021), 295-309 https://doi.org/10.1007/s00280-020-04181-2

[23] Jean Gonfi M. Mvondo, Aristote Matondo, Dani T. Mawete, Sylvie-Mireille N. Bambi, Blaise M. Mbala, Pierre O. Lohohola, In Silico ADME/T Properties of Quinine Derivatives using SwissADME and pkCSM Webservers, *International Journal of TROPICAL DISEASE & Health*, 42, 11, (2021), 1–12 https://doi.org/10.9734/ijtdh/2021/v42i1130492

[24] Md. Ruhul Amin, Farhana Yasmin, Mohammed Anowar Hosen, Sujan Dey, Shafi Mahmud, Md. Abu Saleh, Talha Bin Emran, Imtiaj Hasan, Yuki Fujii, Masao Yamada, Yasuhiro Ozeki, Sarkar Mohammad Abe Kawsar, Synthesis, Antimicrobial, Anticancer, PASS, Molecular Docking, Molecular Dynamic Simulations & Pharmacokinetic Predictions of Some Methyl β -D-Galactopyranoside Analogs, *Molecules*, 26, 22, (2021), 7016 https://doi.org/10.3390/molecules26227016

[25] Innokenty M. Mokhosoev, Dmitry V. Astakhov, Alexander A. Terentiev, Nurbubu T. Moldogazieva, Human Cytochrome P450 Cancer-Related Metabolic Activities and Gene Polymorphisms: A Review, *Cells*, 13, 23, (2024), 1958 https://doi.org/10.3390/cells13231958

[26] Olubadewa A. Fatunde, Sherry-Ann Brown, The Role of CYP450 Drug Metabolism in Precision Cardio-Oncology, *International Journal of Molecular Sciences*, 21, 2, (2020), 604 https://doi.org/10.3390/ijms21020604

[27] Noureddine Mhadhbi, Noureddine Issaoui, Walid S. Hamadou, Jahoor M. Alam, Abdelmonein S. Elhadi, Mohd Adnan, Houcine Naïli, Riadh Badraoui, Physico-Chemical Properties, Pharmacokinetics, Molecular Docking and In-Vitro Pharmacological Study of a Cobalt (II) Complex Based on 2-Aminopyridine, 7, 3, (2022), e202103592 https://doi.org/10.1002/slct.202103592

[28] Imad Ahmad, Aleksey E. Kuznetsov, Abdul Saboor Pirzada, Khalaf F. Alsharif, Maria Daglia, Haroon Khan, Computational pharmacology and computational chemistry of 4-hydroxyisoleucine: Physicochemical, pharmacokinetic, and DFT-based approaches, *Frontiers in Chemistry*, Volume 11 - 2023, (2023), https://doi.org/10.3389/fchem.2023.1145974

[29] P. H. Riyadi, Romadhon, I. D. Sari, R. A. Kurniasih, T. W. Agustini, F. Swastawati, V. E. Herawati, W. A. Tanod, SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in *Spirulina platensis*, *IOP Conference Series: Earth and Environmental Science*, 890, (2021), 012021 https://doi.org/10.1088/1755-1315/890/1/012021

[30] Simone Q. Pantaleão, Philipe O. Fernandes, José Eduardo Gonçalves, Vinícius G. Maltarollo, Kathia Maria Honorio, Recent advances in the prediction of pharmacokinetics properties in drug design studies: a review, *ChemMedChem*, 17, 1, (2022), e202100542 https://doi.org/10.1002%2Fcmdc.202100542

[31] Imad Ahmad, Haroon Khan, Goncagül Serdaroğlu, Physicochemical properties, drug likeness, ADMET, DFT studies, and in vitro antioxidant activity of oxindole derivatives, *Computational Biology and Chemistry*, 104, (2023), 107861 https://doi.org/10.1016/j.compbiolchem.2023.107861

[32] Giuseppina Ioele, Martina Chieffallo, Maria Antonietta Occhiuzzi, Michele De Luca, Antonio Garofalo, Gaetano Ragno, Fedora Grande, Anticancer Drugs: Recent Strategies to Improve Stability Profile, Pharmacokinetic and Pharmacodynamic Properties, *Molecules*, 27, 17, (2022), 5436 https://doi.org/10.3390/molecules27175436

[33] Hiroshi Komura, Reiko Watanabe, Kenji Mizuguchi, The Trends and Future Prospective of In Silico Models from the Viewpoint of ADME Evaluation in Drug Discovery, *Pharmaceutics*, 15, 11, (2023), 2619 https://doi.org/10.3390/pharmaceutics15112619

[34] Viktor Hlaváč, Radka Václavíková, Veronika Brynychová, Pavel Ostašov, Renata Koževnikovová, Katerina Kopečková, David Vrána, Jiří Gatěk, Pavel Souček, Role of Genetic Variation in Cytochromes P450 in Breast Cancer Prognosis and Therapy Response, *International Journal of Molecular Sciences*, 22, 6, (2021), 2826 https://doi.org/10.3390/ijms22062826

[35] Olga Obrezanova, Artificial intelligence for compound pharmacokinetics prediction, *Current Opinion in Structural Biology*, 79, (2023), 102546 https://doi.org/10.1016/j.sbi.2023.102546

[36] Lana Danial Lark Amoa, Belinda Mensah, Younis Sutari, Pharmacogenomics in Anesthesia: Tailoring Anesthetic Agents to Genetic Variations, *Journal of Surgical Case Reports and Images*, 8, 1, (2025), 2690-1897 https://doi.org/10.31579/2690-1897/228