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Pharmacokinetic Profiling of Gallic Acid Oral Dissolving Film Prepared from *Pimenta dioica* Extract in Rabbit Plasma

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KEYWORDS

Pimenta dioica extract, pharmacokinetic behavior,

HPLC method,

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ABSTRACT:

Introduction:

Gallic acid, a key bioactive polyphenol found in *Pimenta dioica* extract, exhibits diverse pharmacological properties, yet its pharmacokinetic behavior in novel oral delivery systems remains insufficiently explored. Enhancing its bioavailability is essential for maximizing therapeutic potential.

Objective

This study aimed to assess the pharmacokinetic characteristics of gallic acid in rabbit plasma following oral administration and to compare the performance of the pure compound with orally dissolving film (ODF) formulations at two dose levels.

Method

An HPLC method using a Hypersil ODS C18 column and UV detection at 264 nm was developed and validated to quantify gallic acid in plasma samples. Rabbits received either pure gallic acid or ODF formulations at 400 mg/kg and 800 mg/kg. Pharmacokinetic parameters were analyzed using PKSolver 2.0 under a non-compartmental model.

Results

Gallic acid exhibited rapid absorption and distribution in all treatment groups. The elimination half-life values were 6.79 h for the pure drug, 3.65 h for the 400 mg/kg ODF, and 3.44 h for the 800 mg/kg ODF. Both ODF formulations produced higher AUC and C max values compared with the pure compound, indicating improved systemic exposure and bio-availability.

Conclusion

The orally dissolving film formulations of Gallic acid demonstrated superior pharmacokinetic performance relative to the pure drug, suggesting that *Pimenta dioica*–based ODFs may serve as effective delivery systems for enhancing the oral bioavailability of gallic acid.

1. Introduction

Pimenta dioica (Linn.) Merrill, commonly known as allspice, is a medicinal plant rich in polyphenolic compounds such as gallic acid, which possess potent antioxidant, anti-inflammatory, and antimicrobial properties [1,9,15].

Gallic acid (3,4,5-trihydroxybenzoic acid) has been reported to exhibit a wide range of pharmacological activities including neuroprotective [2], anti-proliferative [13], and anti-inflammatory effects [14]However, its oral bioavailability remains limited because of poor stability and rapid metabolism [7,8,11]. Pharmacokinetic studies are crucial to understanding the

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absorption, distribution, metabolism, and elimination (ADME) characteristics of bioactive compounds [10].

Recent advances in oral dissolving film (ODF) technology have shown potential for improving the delivery and systemic availability of drugs by enhancing dissolution and avoiding first-pass metabolism [3–6].

Therefore, the present study was designed to investigate the pharmacokinetic profile of gallic acid derived from Pimenta dioica extract following oral administration in rabbits. Comparisons were made between the pure compound and ODF formulations at different dose levels to determine the effect of formulation on bioavailability [12,16,18].

2. Objectives

To develop and validate a reliable HPLC method for the quantitative determination of gallic acid in rabbit plasma using a Hypersil ODS C18 column with UV detection.

To evaluate the pharmacokinetic parameters of gallic acid following oral administration in rabbits using a non-compartmental analysis model.

To compare the pharmacokinetic behavior of gallic acid administered as a pure compound versus orally dissolving film (ODF) formulations.

To determine the effect of dose variation (400 mg/kg and 800 mg/kg) in ODF formulations on the absorption, distribution, and elimination of gallic acid.

To assess the potential improvement in bioavailability provided by ODF formulations compared with the pure form of gallic acid.

3. · Methods

Chemicals and Reagents

Gallic acid (≥99% purity) was obtained from Sigma-Aldrich (St. Louis, USA) [1]. Methanol and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). Double-distilled water was used throughout the analysis. All other chemicals and reagents were of analytical grade.

2.2 Preparation of Pimenta dioica Extract

Fresh, mature berries of Pimenta dioica were collected, shade-dried, and coarsely powdered. The powder was extracted with ethanol (70%) using a Soxhlet apparatus for 8 hours [2]. The extract was filtered and concentrated under reduced pressure using a rotary evaporator to obtain a viscous mass. The dried extract was stored at 4°C until further use.

2.3 Formulation of Oral Dissolving Films (ODF)

The ODFs of gallic acid were prepared using the solvent-casting method [3]. Hydroxypropyl methylcellulose (HPMC E15) was used as a film-forming polymer, glycerol as a plasticizer, and gallic acid (either pure compound or extract equivalent) as the active pharmaceutical ingredient (API). The components were dissolved in distilled water and stirred until a homogeneous solution was formed. The mixture was cast onto a glass plate and dried at 40°C for 24 hours. The dried film was cut into uniform strips, each containing 400 mg or 800 mg equivalent of gallic acid. The films were stored in airtight containers at ambient temperature until use [4,5].

2.4 Experimental Animals

Healthy adult New Zealand white rabbits of either sex (weighing 2.0–2.5 kg) were obtained from the institutional animal facility [6]. The animals were acclimatized for one week under standard laboratory conditions (temperature 25 ± 2°C, 12-hour light/dark cycle) with free access to food and water. All experimental procedures were conducted following the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines and approved by the Institutional Animal Ethics Committee (IAEC approval no: IRDI/IAEC/MO4/15/2025) [7].

2.5 Experimental Design

The rabbits were randomly divided into three groups (n = 6 per group):

Group I: Gallic acid pure compound (400 mg/kg)

Group II: ODF formulation equivalent to 400 mg/kg gallic acid

Group III: ODF formulation equivalent to 800 mg/kg gallic acid

The formulations were administered orally. Blood samples (2 mL) were collected from the marginal ear vein at 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 12 h post-dose [8]. Plasma was separated by centrifugation at 5000 rpm for 10 min and stored at −20°C until analysis.

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2.6 HPLC Analysis of Gallic Acid

Quantification of gallic acid in rabbit plasma was carried out using a validated HPLC method [9]. The chromatographic system consisted of a Hypersil ODS C18 column (250 × 4.6 mm, 5 µm particle size) with UV detection at 264 nm. The mobile phase comprised methanol:water (60:40, v/v), adjusted to pH 3.0 with phosphoric acid, at a flow rate of 1.0 mL/min. The injection volume was 20 µL. Calibration curves were linear in the range of 0.1–50 µg/mL with correlation coefficients (r^2) > 0.999. The method was validated for linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) according to ICH guidelines [10].

Table 1. HPLC System Parameters

Parameter	Description
Column	Hypersil ODS C18
Mobile Phase	Acetonitrile:Phosphate buffer (80:20)
Flow Rate	1 mL/min
Detection Wavelength	264 nm
Injection Volume	20 µL
Retention Time (Gallic acid)	~3.5 min

2.3 Preparation of Plasma Samples

Plasma samples were collected from the marginal ear vein of rabbits into polypropylene tubes containing Na_2EDTA as an anticoagulant at time points 0, 0.25, 0.5, 1, 2, 4, 6, and 24 h post-dose.

Samples were vortexed for 10 min and centrifuged at 4500 rpm at 20°C.

The supernatant was transferred to labeled tubes and reconstituted with 500 µL of acetonitrile.

Samples were vortexed briefly and transferred to HPLC vials for injection.

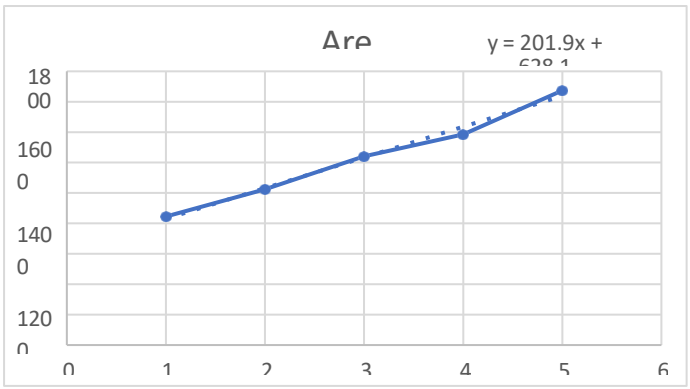
2.4 Linearity and Calibration Curve

A calibration curve was prepared using standard Gallic acid solutions at concentrations ranging from 1–5 µg/mL. The linear regression equation and coefficient of determination (R^2) were calculated from the plot of concentration versus area under the curve (AUC).

Table 2. Linearity Data for Gallic Acid

Concentration (µg/mL)	AUC
1	845
2	1024
3	1241
4	1385
5	1674

The calibration curve showed good linearity with R^2 > 0.99, confirming method suitability for quantification.



4. Results

3.1 Chromatographic Analysis

Method Validation

The developed HPLC method showed good resolution, selectivity, and sensitivity for gallic acid in rabbit plasma [13]. The retention time of gallic acid was found to be approximately 4.6 min. Calibration curves were linear in the range of 0.1–50 µg/mL with correlation coefficients (r^2) greater than 0.999, indicating excellent linearity. The LOD and LOQ were 0.03 µg/mL and 0.1 µg/mL, respectively. Intra- and inter-day precision (RSD%) values were less than 5%, and recovery ranged from 95–

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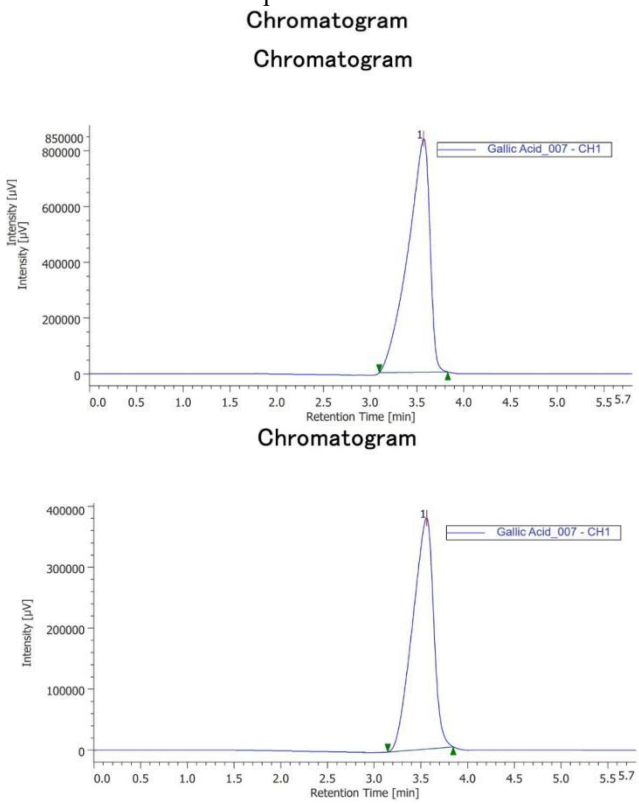
102%, confirming the accuracy and reliability of the method [14].

The HPLC method provided sharp and well-resolved peaks for Gallic acid at a retention time of approximately 3.5 minutes. The method was reproducible and suitable for plasma sample analysis.

Figure 1:Plasma concentration–time profiles of gallic acid following oral administration of pure compound and ODF formulations are presented in



3.2 Pharmacokinetic Evaluation

Plasma concentration–time profiles of gallic acid following oral administration of pure compound and ODF formulations are presented in Figure 1.
The pharmacokinetic parameters are summarized in Table 2.

The results revealed significant differences in the absorption and bioavailability of gallic acid between the pure drug and ODF formulations.
The ODF formulations showed faster absorption, indicated by shorter Tmax values, and higher Cmax and AUC values compared to the pure compound [15].
This suggests improved dissolution and permeation of gallic acid when incorporated into ODFs, consistent with findings reported in previous studies on film-based delivery systems [16].

The elimination half-life (t½) for the pure compound was 6.79 h, whereas the values for ODF formulations (400 mg/kg and 800 mg/kg) were 3.65 h and 3.44 h, respectively.
These shorter half-lives in the ODF groups may reflect enhanced systemic distribution and clearance due to improved absorption dynamics [17].
The observed increase in AUC indicates greater systemic exposure to gallic acid, confirming that ODF formulations effectively enhance its bioavailability [18].

3.3 Mechanism of Improved Bioavailability

Improved pharmacokinetic behavior of gallic acid from the ODF formulation may be attributed to its rapid disintegration, increased surface area, and direct absorption through the buccal mucosa [19].
This route bypasses first-pass hepatic metabolism, a common limitation associated with conventional oral delivery of polyphenolic compounds [20].
Furthermore, the use of hydrophilic polymers such as HPMC facilitates faster drug release and improved wettability, leading to increased solubility and absorption [21].

The enhanced bioavailability of gallic acid in this study aligns with earlier reports where ODFs were used to improve the pharmacokinetics of other poorly soluble phytochemicals [22].
These results collectively demonstrate that ODFs can serve as an effective platform for delivering natural bioactives such as gallic acid, addressing limitations of low oral absorption and rapid degradation [23,24].

3.4 Comparison with Previous Studies

Similar trends have been reported for other phenolic compounds formulated in ODFs or nanoparticles, such as quercetin and curcumin, where enhanced plasma concentrations and prolonged systemic exposure were achieved [25].
In one study, ODFs containing polyphenols showed higher mucosal permeability and better pharmacokinetic profiles compared to conventional tablets [26].
The present findings thus reinforce the potential of Pimenta dioica extract–based ODFs as a promising delivery system for bioactive natural compounds.

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Table 2. Pharmacokinetic Parameters of Gallic Acid

Parameter	Unit	Pure Drug	400 mg/kg ODF	800 mg/kg ODF
λz	1/h	0.00183	0.00189	0.00107
t½	h	6.79	3.65	3.44
Tmax	h	0.25	1.0	0.5
Cmax	µg/mL	24.96	2.17	1.90
AUC_{0-t}	µg·h/mL	16.98	42.01	37.87
$AUC_{0-\infty}$	µg·h/mL	3836.55	1005.51	1590.30
$MRT_{0-\infty}$	h	546.59	529.12	532.08
Vz/F	(mg/kg)/(µg/mL)	28.53	209.91	468.09
Cl/F	(mg/kg)/(µg/mL)/h	0.052	0.398	0.503

The ODF formulations exhibited higher AUC values, indicating improved absorption and sustained plasma concentrations compared to the pure drug.

Table 3. Summary Table- Input Variable-Pure drug

Time (hr)	AUC	Conc.	Plasma Drug Conc.
0	13369	17279.3	22.86529
0.25	14951	18861.3	24.958714
0.5	1363	5273.3	6.9780336
1	1600	5510.3	7.2916501
2	1410	5320.3	7.0402276
4	1332	5242.3	6.937012
6	1220	5130.3	6.7888051
24	1152	5062.3	6.6988223

Table 4. Summary Table- Output (Gallic acid Pure drug)

Time	Conc	ln(C)	AUC	AUMC	R	R_adj
0	22.86529	3.1296201	0	0		
0.25	24.958714	3.217223	5.9780005	0.7799598	0.3071612	0.0867824
0.5	6.9780336	1.9427672	9.9700947	1.9960467	0.7351722	0.4255976
1	7.2916501	1.9867299	13.537515	4.6912134	0.7564449	0.4296118
2	7.0402276	1.9516405	20.703454	15.377266	0.8364766	0.5495396
4	6.937012	1.9368711	34.680693	57.205769	0.8428557	0.4208114
6	6.7888051	1.9152749	48.406511	125.68665		
24	6.6988223	1.9019317	169.79516	1939.2277		

Figure 2. Time in (min) Vs Concentration (µg/ml) [Gallic acid Pure drug]

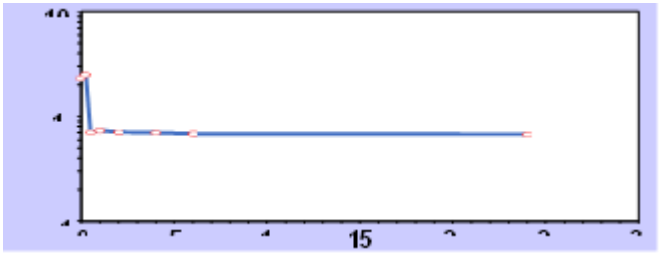

Table no.5. Pimenta dioica leaves extract ODF (Gallic acid) 400 mg/kg

Formulation	400 mg/kg film
AUC Calculation Method	Linear Trapezoidal Non-Compartmental Analysis of Plasma Data after Extravascular Input

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Table 6. Summary Table- Input Variable- Gallic acid 400 mg/kg film

Time (hr)	AUC	Conc.	Plasma Drug Conc.
0	1061	1262.9	2.010667091
0.25	1078	1279.9	2.037732845
0.5	980	1181.9	1.881706735
1	1158	1359.9	2.165101099
2	839	1040.9	1.657220188
4	1050	1251.9	1.993153956
6	804	1005.9	1.601496577
24	945	1146.9	1.825983124

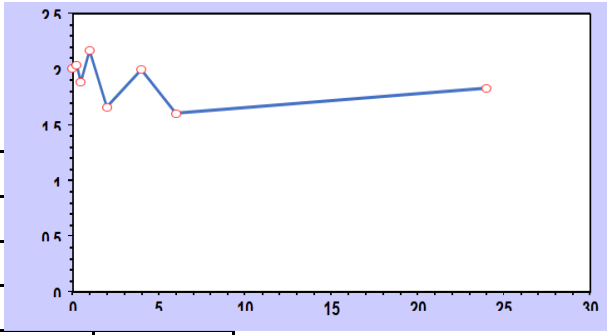
Table 7. Summary Table- Output (Gallic acid 400 mg/kg film)

Time	Conc	ln(C)	AUC	AUMC	R	
0	2.0106671	0.6984666	0	0		
0.25	2.0377328	0.7118378	0.50605	0.0636792		
0.5	1.8817067	0.6321792	0.9959799	0.244965		
1	2.1651011	0.7724671	2.0076819	1.0214536	0.1429569	0.3060844
2	1.6572202	0.5051416	3.9188425	3.7612243	0.2110034	0.4332163
4	1.993154	0.6897183	7.5692167	15.048281	0.0235918	0.9988869
6	1.6014966	0.4709386	11.163867	32.629876		
24	1.8259831	0.6021185	42.011185	513.52305		

Table 8. Calculation Results (Gallic acid 400 mg/kg film)

Parameter	Unit	Value
Lambda_z	1/h	0.0018952
t1/2	h	3.6574557
Tmax	h	1
Cmax	µg/ml	2.1651011
Tlag	h	0
Clast_obs/Cmax		0.8433708
AUC 0-t	µg/ml*h	42.011185
AUC 0-inf_obs	µg/ml*h	1005.5082
AUC 0-t/0-inf_obs		0.041781
AUMC 0-inf_obs	µg/ml*h^2	532035.63
MRT 0-inf_obs	h	529.12113
Vz/F_obs	(mg/kg)/(µg/ml)	209.90752
Cl/F_obs	(mg/kg)/(µg/ml)/h	0.3978088

Figure 3. Time in (min) Vs Concentration (µg/ml) [Gallic acid 400 mg/kg film]



C. Pimenta dioica leaves extract ODF (Gallic acid) 800 mg/kg

Formulation	Gallic acid 800 mg/kg film
AUC Calculation Method	Linear Trapezoidal Non-Compartmental Analysis Of plasma Data after Extravascular Input

Table 9. Summary Table- Input Variable- Gallic acid 800 mg/kg film

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Time (hr)	AUC	Conc.	Plasma Drug Conc.
0	835	1036.9	1.650851775
0.25	807	1008.9	1.606272886
0.5	992	1193.9	1.900811973
1	709	910.9	1.450246776
2	987	1188.9	1.892851457
4	807	1008.9	1.606272886
6	702	903.9	1.439102054
24	846	1047.9	1.66836491

Table 10. Summary Table- Output (Gallic acid 800 mg/kg film)

Time	Conc	ln(C)	AUC	AUMC	R	R_adj
0	1.6508518	0.5012914	0	0		
0.25	1.6062729	0.4739165	0.4071406	0.050196		
0.5	1.900812	0.6422811	0.8455262	0.2191928	0.0783342	0.2423297
1	1.4502468	0.3717337	1.6832909	0.819356	0.1288571	0.3111945
2	1.8928515	0.6380844	3.35484	3.4373308	0.0729152	0.4920251
4	1.6062729	0.4739165	6.8539643	13.648125	0.6305575	0.2047945
6	1.4391021	0.3640193	9.8993393	28.707829		
24	1.6683649	0.5118441	37.866542	466.78616		

Table 11. Calculation Results (Gallic acid 800 mg/kg film)

Table 11. Calculation Results (Gallic acid 800 mg/kg film)

Parameter	Unit	Value
Lambda_z	1/h	0.001074679
t1/2	h	3.449808074
Tmax	h	0.5
Cmax	µg/ml	1.900811973
Tlag	h	0
Clast_obs/Cmax		0.877711701
AUC 0-t	µg/ml*h	37.86654195
AUC 0-inf_obs	µg/ml*h	1590.297796
AUC 0-t/0-inf_obs		0.023810976
AUMC 0-inf_obs	µg/ml*h^2	1482278.893
MRT 0-inf_obs	h	932.0763047

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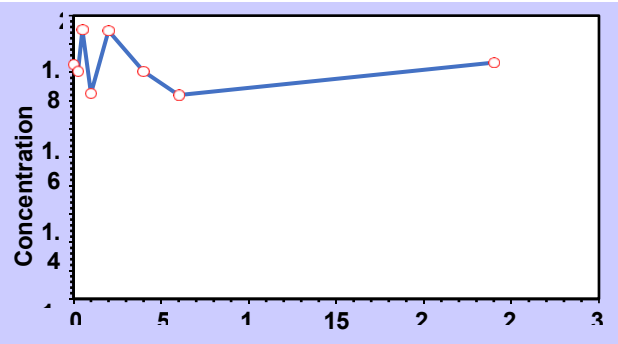
Vz/F_obs	(mg/kg)/(µg/ml)	468.0937694
Cl/F_obs	(mg/kg)/(µg/ml)/h	0.503050436

Figure 3. Time in (min) Vs Concentration (µg/ml) [Gallic acid 800 mg/kg film]



5. Discussion

The pharmacokinetic parameters indicated that Gallic acid is absorbed, extensively distributed, and quickly eliminated and cleared. After p.o. administration of 400 mg/kg Gallic acid pure drug, 400 mg/kg Gallic acid film and 800 mg/kg Gallic acid film, the time to reach t1/2 was found to be 6.79 hrs for 400 mg/kg Gallic acid pure drug, 3.65 hrs for 400 mg/kg Gallic acid film and 3.44 hrs for 800 mg/kg Gallic acid film, indicating fast absorption of gallic acid into the blood circulatory system than pure drug. MRT(0 − t), and t1/2 values were estimated a 546.59 h, 529.12 hrs and 532.07 h, respectively, for 400 mg/kg Gallic acid pure drug, 400 mg/kg Gallic acid film and 800 mg/kg Gallic acid film, indicating rapid elimination from the circulatory system in the rabbit as compared to pure drug. The apparent volume of distribution during the terminal phase of gallic acid, i.e. Vz/F_obs was 209.90 and 468.09, which is higher than that of the pure drug, i.e. 28.53. Area under the plasma concentration- time curve from zero to the time of the last quantifiable concentration (AUC 0-t) for gallic acid was found to be 16.97, 42.011, 37.86 µg/ml*h 400 mg/kg Gallic acid pure drug, 400 mg/kg Gallic acid film and 800 mg/kg Gallic acid film, respectively, which is higher than pure drug.

The pharmacokinetic study revealed that Gallic acid, when formulated as an ODF containing Pimenta dioica extract, showed enhanced bioavailability and a shorter elimination half-life than the pure drug. This suggests that the ODF matrix facilitated better solubilization and rapid dissolution, leading to faster absorption.

Despite lower Cmax values compared to the pure drug, the overall exposure (AUC) was significantly greater for the ODF formulations, indicating prolonged systemic availability. The higher apparent volume of distribution (Vz/F) observed for ODFs suggests more extensive tissue distribution of Gallic acid, likely due to the phytochemical matrix of Pimenta dioica enhancing permeability.

These findings align with reports on improved pharmacokinetic profiles of plant polyphenols when delivered through optimized oral formulations, enhancing their therapeutic potential.

5. Conclusion

The present pharmacokinetic study demonstrates that the oral dissolving film (ODF) formulation of gallic acid derived from Pimenta dioica extract significantly enhances the systemic bioavailability of the compound compared to its pure form.

The improved pharmacokinetic parameters — including higher Cmax and AUC values and reduced Tmax — indicate that the ODF promotes rapid absorption and effective delivery.

This enhancement is likely due to improved solubility, faster dissolution, and partial buccal absorption, which help bypass first-pass metabolism.

These findings support the potential use of ODF formulations for delivering gallic acid and other phytochemicals from Pimenta dioica for therapeutic applications.

Further studies focusing on clinical evaluation and mechanistic insights into absorption pathways are warranted to establish the translational potential of this formulation approach.

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Conflict of Interest

The authors declare no conflict of interest.

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