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Drying Method Selection as a Critical Determinant of Pharmaceutical Quality in Biotherapeutic *Lucilia sericata* Larval Extracts

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Abstract

Objectives: This study aimed to compare the preservation efficacy of protein content and antioxidant activity in crude whole-body larval extract (WBE) products obtained from *Lucilia sericata* second instar (L2) larvae using four different drying protocols. **Methods:** The larvae were processed using lyophilisation (freeze-drying), infrared drying, microwave drying, and conventional oven drying methods. The antioxidant capacities (using the DPPH radical scavenging and FRAP iron reduction methods) and total protein concentrations (using the Bradford and Lowry methods) of the resulting extracts were then analysed. Statistical analyses (one-way ANOVA, Tukey HSD test, $p<0.05$) revealed that drying methods had a highly significant effect on the results. **Findings:** The highest antioxidant activity (DPPH: IC_{50}: 0.1350 $\pm$ 0.0242 mg/mL; FRAP: 45.4430 $\pm$ 0.0037 mg TE/g) and protein content (237.1 $\pm$ 0.0203 mg/ BSA eq/g dry weight (Bradford method), 164.8125 $\pm$ 0.0056 mg/BSA eq/g dry weight (Lowry method)) were observed in the lyophilisation group. This group was followed by infrared drying, which showed moderate values (DPPH: IC50: 0.2546 $\pm$ 0.0341 mg/mL; FRAP: 40.1603 $\pm$ 0.0082 mg TE/g; Protein: 173.0378 $\pm$ 0.0060 mg/BSA eq/g dry weight (Bradford method), 144.4583 $\pm$ 0.0065 mg/ BSA eq/g dry weight (Lowry method), followed by oven drying with significantly lower values, and microwave drying with the lowest bioactivity. The findings clearly demonstrate that the duration and intensity of thermal stress have a direct degradative effect on heat-sensitive proteins and antioxidant molecules in the larvae. **Novelty**: Drying methods have a significant effect on the bioactivity of larval secretions and have implications for Larva Debridement Therapy (LDT) applications. The stability of bioactive compounds responsible for wound healing and antimicrobial properties is critical in these products. By demonstrating that lyophilisation preserves up to four times more protein content and significantly higher antioxidant capacity than conventional thermal methods, this research offers a standardized, evidence-based protocol for the industrial-scale production of high-quality, bioactive larval extracts. This advancement directly addresses the critical bottleneck of biomolecule degradation during processing, paving the way for more stable, accessible, and potentially efficacious pharmaceutical products for LDT; confirmation of therapeutic efficacy will require further functional, antimicrobial, and in vivo assays.

Keywords: *Lucilia sericata*; biotherapy; drying optimisation; thermal degradation; antioxidant capacity; protein stabilisation; lyophilisation; infrared drying

1 Introduction

Chronic, non-healing wounds — including diabetic foot ulcers, venous leg ulcers, and pressure injuries — represent a growing global healthcare burden, characterised by a self-perpetuating cycle of sustained inflammation, excessive reactive oxygen species (ROS) accumulation, and colonisation by antibiotic-resistant bacterial biofilms, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*[1],[2]. Conventional treatment modalities frequently fail in this context, leaving a critical therapeutic gap that demands biologically targeted alternatives.

Larval Therapy (LT), employing *Lucilia sericata* (Diptera: Calliphoridae) larvae, has re-emerged as a clinically validated intervention for complex wounds, encompassing enzymatic debridement, direct antimicrobial action against drug-resistant pathogens, and stimulation of granulation tissue[3],[4]. The therapeutic potency of LT resides in the excretion/secretion (ES) complex of second-instar (L2) larvae — a biochemically rich mixture of serine proteases, metalloproteases, antimicrobial peptides (AMPs), growth factors, and diverse antioxidant molecules[5]. Larval antioxidants are particularly crucial for neutralizing the excessive ROS burden in the wound microenvironment, interrupting the oxidative-stress cycle that perpetuates chronic inflammation[2]. Furthermore, the biochemical profile of these bioactives is significantly influenced by the larvae's developmental stage and nutritional substrate[6]. Despite compelling efficacy data, LT adoption remains constrained by patient aversion to live larvae, logistical complexities of delivering sterile organisms, and the absence of standardized, shelf-stable pharmaceutical formats[3],[7]. These limitations have driven pharmaceutical research toward the development of standardized, storable larval extracts or powders. Hoff et al. (2025) demonstrated that a lyophilized *L. sericata* extract produced under GMP conditions achieved significant fibrin reduction and wound closure in clinical trials[8] — yet this study did not compare drying methods or empirically justify the choice of lyophilisation over alternatives. Sherafati et al. (2022) documented the broad therapeutic effects of larval ES products in vivo[3], while Marzan et al. (2026) confirmed the antibacterial and antioxidant potential of *L. sericata* extract for biomedical applications[5], and Giacaman et al. (2022) integrated larval ES into bioactive electrospun scaffolds[9]. Critically, none of these works addressed the fundamental question of how the choice of drying method affects bioactive preservation in *L. sericata* crude whole-body L2 larval extract (WBE).

The drying process is a critical quality attribute (CQA) in the production of larval extracts, as applied thermal energy can irreversibly trigger protein denaturation, Maillard crosslinking, and oxidative degradation of antioxidant compounds[10],[11]. While lyophilisation preserves thermolabile biopharmaceuticals via sublimation[10],[12], and alternative methods—infrared, microwave, and conventional oven drying—offer varying degrees of rapid moisture removal or economic feasibility, their comparative impact on the antioxidant-rich, multi-component ES of *L. sericata* L2 larvae remains unexplored. Although studies on other species like *Tenebrio molitor* suggest freeze-drying yields lower denaturation than thermal modalities[13], this research gap directly impedes the development of pharmaceutical-grade manufacturing protocols[14],[15]. The present study addresses this deficiency through a rigorous biochemical comparison of these four drying techniques, quantifying antioxidant capacity (DPPH, FRAP) and total protein content (Bradford, Lowry) to test the hypothesis that lyophilisation's elimination of thermal and oxidative stresses ensures superior preservation of structural and functional integrity. By establishing an evidence-based protocol for industrial-scale production, this research aims to advance the translation of maggot-derived biotherapeutics into accessible pharmaceutical products for regenerative medicine.

2 Methodology

2.1 Larvae Culture and Secretion Collection

The *Lucilia sericata* population was obtained from the entomology laboratory of the Department of Traditional Complementary Integrative Medicine at Ankara Yıldırım Beyazıt University. The fly colony was maintained under laboratory conditions in a controlled environment chamber (25±2°C, 60±5% relative humidity, 12:12 hours light:dark photoperiod) and fed a standard diet containing sugar, milk powder, and subsequently beef liver. Sterile eggs were placed on autoclaved bovine liver, and L2 larvae (mid-feeding stage, approximately 48-60 hours later) were manually collected according to morphological criteria[14]. The collected second-stage larvae were sterilised for one minute in sterile distilled water followed by a 0.5% sodium hypochlorite solution to remove surface food debris and microbial load, then rinsed three times with sterile distilled water[15]. Excess moisture was removed on sterile filter paper, and four equal homogeneous groups of approximately 10 grams (fresh weight) were formed.

2.2 Applied Drying Protocols

Each group was subjected to one of the four drying methods detailed below. All drying processes were terminated based on the principle of the sample reaching a constant weight (mass change <0.1%). All dried samples were homogenised in a grinder (Retsch MM 400) in liquid nitrogen to produce a fine powder and stored in a desiccator at +4°C until analysis.

2.2.1. Lyophilisation (L)

Samples were first completely frozen at -80°C in an ultra-freezer (Thermo Scientific) for 24 hours. The frozen larvae were transferred to the shelves of a pre-cooled (-50°C) lyophiliser (Christ Alpha 1-4 LDplus). Primary drying was carried out for 48 hours under a vacuum of 0.011 mBar, with the condenser temperature set to -55°C. During the secondary drying stage, the shelf temperature was gradually increased to +20°C, and the process was continued for an additional 12 hours [16].

2.2.2. Infrared Drying (IR)

An infrared dryer (hair dryer type, operating temperature: 30–80°C) was used. The larvae were placed in a single layer with a distance of 15 cm between the infrared lamps and the sample. The drying temperature was maintained at 45±5°C, and moisture removal was achieved using the dryer's internal fan (air flow rate 1.5 m/s). The process lasted approximately 8 hours [17].

2.2.3. Microwave Drying (M)

Samples were spread in 10 g portions in a glass Petri dish and dried in a microwave oven (Arçelik) with a nominal power of 800W. Due to the risk of excessive heating from continuous power application, an intermittent drying protocol was applied: cycles of 30 seconds of drying (750W) followed by 60 seconds of waiting (0W) were repeated. The total active drying time was approximately 10 minutes [7], [18]. The dielectric heating mechanism of microwave drying, while rapid, can induce uneven temperature distributions and activate degradative enzymes in biological matrices [18].

2.2.4. Oven Drying (E)

The larvae were placed in a single layer on aluminium foil-lined trays and dried in an air-circulating oven (Nüve FN 400) at 60±2°C for 24 hours [19].

2.3 Extraction Procedure

One gram (±0.01 g) of powder from each drying group was transferred into 10 mL of cold (4°C) phosphate-buffered saline (PBS, 0.1 M, pH 7.4). The mixture was homogenized using a homogenizer (IKA T18 digital Ultra-Turrax) at 4°C for 2 minutes. The resulting suspension was centrifuged at 15,000 × g for 20 minutes at 4°C (Hettich Mikro 220R). The clear supernatant was collected into sterile tubes and used for subsequent antioxidant and protein analyses.

2.4 Biochemical Analyses

2.4.1. Determination of Total Antioxidant Capacity

2.4.1.1. DPPH Radical Scavenging Activity . The free radical scavenging effects of secretion extracts obtained from *Lucilia sericata* larvae were measured by their ability to decolourise the violet/purple colour of the stable radical. This method involves measuring the colour formed by the interaction of the extracts with the DPPH free radical at 517 nm, and comparing the result with that obtained using a standard substance [20]. For this study, 100 μL of a 100 μM DPPH solution in methanol and 100 μL of larval extracts at various concentrations were mixed together. Following a 30-minute incubation period at room temperature, spectrophotometric absorbance was measured at 517 nm. The DPPH free radical scavenging effect of the extracts was then calculated as a percentage of radical reduction. Each experiment was performed in triplicate. Trolox was used as the reference standard [Figure 1]. The DPPH free radical scavenging activity of the larva extracts was calculated using the following formula:

$$\text{Inhibition } \% = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}/\text{Absorbance}_{\text{Control}}] \times 100$$

A calibration graph was plotted for each extract using the % inhibition values at different concentrations, and the IC_{50} values were calculated. The IC_{50} value for each extract was determined by plotting percentage DPPH inhibition against extract concentration in Microsoft Excel and fitting a linear trendline (y = mx + b) to the resulting data points; the concentration corresponding to 50% inhibition (y = 50) was then solved algebraically from this linear equation.

2.4.1.2. FRAP (Ferric Reducing Antioxidant Power) Activity . To evaluate the antioxidant potential of the secretion extracts, the FRAP (Ferric Reducing Antioxidant Power) assay, which is based on the reduction capacity of ferric (Fe^{3+}) ions, was employed. The protocol described previously by Youn et al. (2019) was used as the basis for this method [21]. According to the experimental protocol, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution prepared in 40 mM hydrochloric acid, and 20 mM ferric chloride ($FeCl_3$) were mixed in a ratio of 10:1:1. Subsequently, 150 μL of FRAP reagent was combined with 20 μL of sample, obtained from serial dilutions of the extracts at a stock concentration of 1000 μg/mL. Following incubation at 37°C in the dark for 30 minutes, the reaction mixture was transferred to a 96-well microplate and absorbance was measured at 595 nm using a microplate reader (Thermo Varioskan Flash Microplate Reader). Trolox (3.90625–250 mg TE/g) was used as the reference standard, and results were expressed as Trolox Equivalent (TE). All samples were analyzed in triplicate, and the results are reported as TE ± standard deviation (SD) derived from three independent experiments [Figure 1].

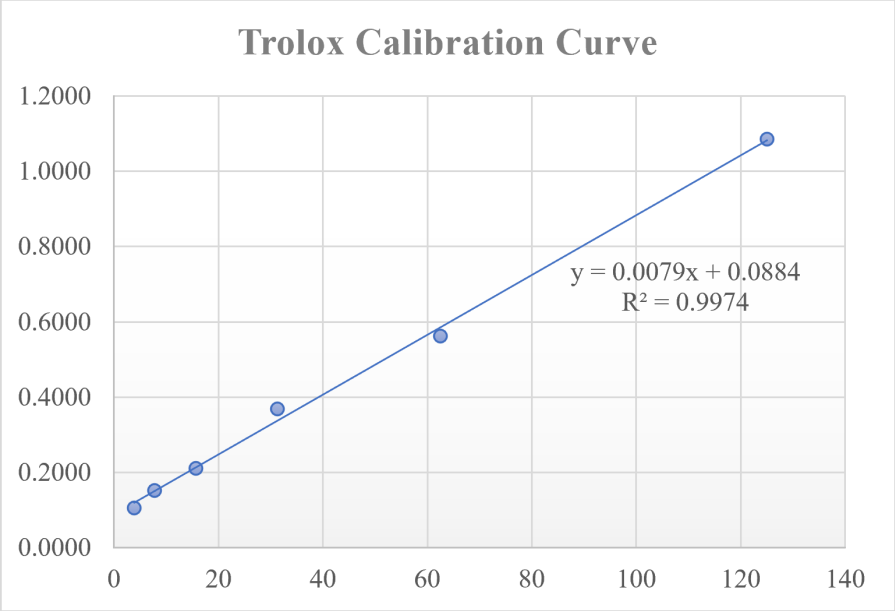

Fig 1. Trolox Calibration Curve

2.4.2. Determination of Total Protein Content

2.4.2.1. Bradford Method. The total protein content in samples and standard solutions was determined using the colorimetric method developed by Bradford [22]. In this method, the interaction of proteins with Coomassie Brilliant Blue dye results in the formation of a characteristic complex that can be measured at 595 nm. For analysis, standards at different concentrations ranging from 7.8125 to 1000 μg/mL were prepared by serial dilutions with distilled water from a bovine serum albumin (BSA) standard solution. A 10 μL volume of each standard and sample solution was transferred in triplicate to a 96-well flat-bottom microplate. Subsequently, 250 μL of pre-prepared Bradford reagent, brought to room temperature, was added to each well. The mixture was gently mixed by pipetting to ensure homogeneity for 30 seconds. The microplate was incubated for 10 minutes at room temperature (22 ± 2 °C) in the dark to allow the reaction to proceed. At the end of the incubation period, the absorbance values of the reaction products were measured at 595 nm using a microplate reader (Thermo Scientific Multiskan). Only the control well containing the solvent and reagent was used as a blank in the measurements, and the net absorbance values were calculated by subtracting the blank value from the absorbance values of all other wells. Based on the results obtained, a standard curve was created, and the protein concentrations of the unknown samples were quantitatively calculated in mg/ BSA eq/g dry weight according to this curve. The standard curve showed a linear relationship with a high correlation coefficient (R^2 = 0.9819) [Figure 2].

2.4.2.2. Lowry Method . The protein content was determined using the colorimetric method described by Lowry et al. [23]. The method is based on a two-step reaction: in the first step, in an alkaline medium, the peptide bonds of proteins form complexes with copper (II) ions, producing reduced copper (I). In the second stage, the Folin–Ciocalteu reagent reacts with aromatic amino acid residues to form a blue-coloured complex. The intensity of the resulting colour is directly proportional

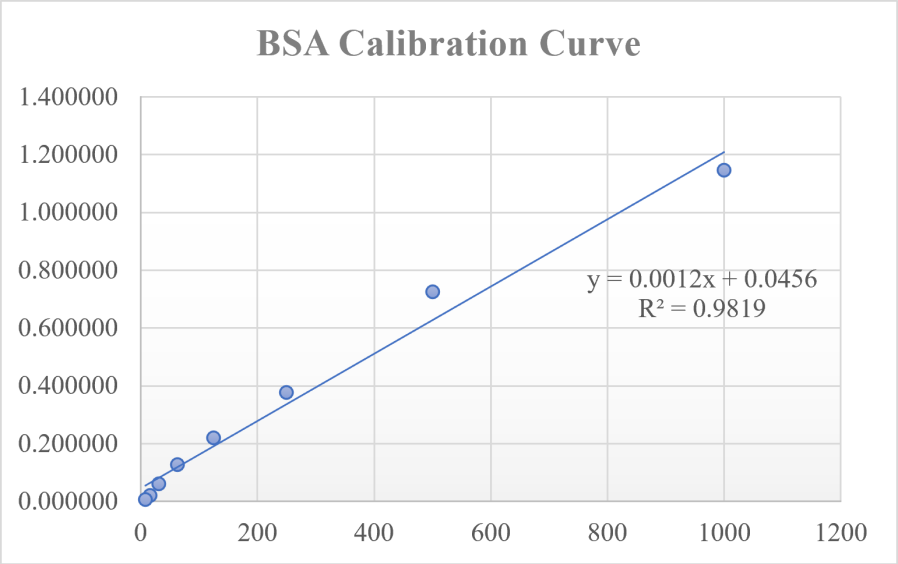

Fig 2. BSA Standard Calibration Curve (Bradford Method)

to the protein concentration and is measured spectrophotometrically at 750 nm. 50 μL of sample or a bovine serum albumin (BSA) standard solution prepared in the range of 7.8125–500 μg/mL was added to each well, followed by 50 μL of alkaline copper reagent, and the mixture was incubated at room temperature for 10 minutes. Subsequently, 25 μL of diluted Folin–Ciocalteu reagent (v/v) was added, and the mixture was left to incubate in the dark for 30 minutes. The absorbance of the blue-coloured complex formed at the end of incubation was measured at 750 nm using a microplate reader. All analyses were performed in triplicate (n = 3); protein concentrations were calculated using the regression equation obtained from the BSA standard curve [Figure 3], and results are expressed as mean $\pm$ standard deviation (Mean $\pm$ SD).

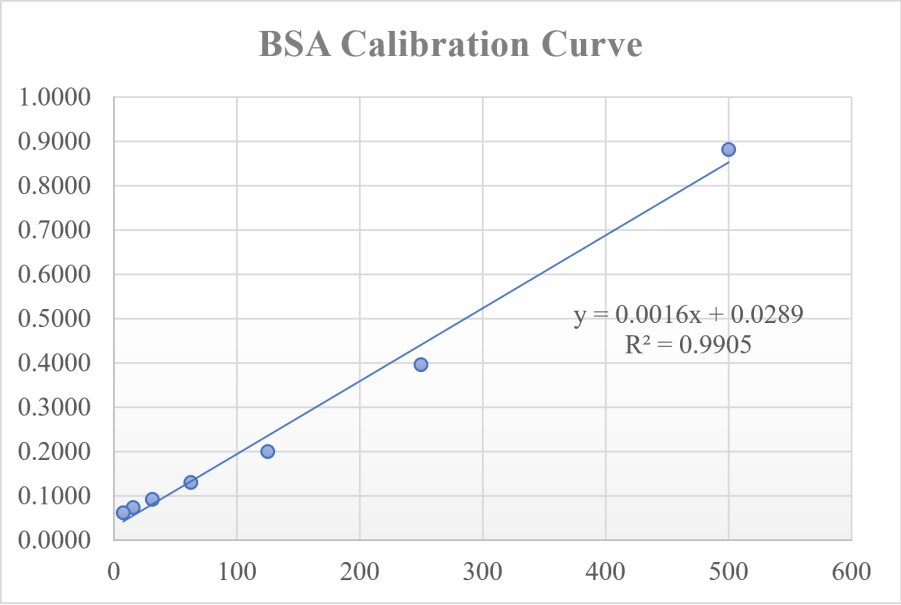

Fig 3. BSA Standard Calibration Curve (Lowry Method)

2.5 Statistical Analysis

All experiments were conducted as independent triplicates. The obtained data were presented as mean ± standard deviation (Mean ± SD). One-way analysis of variance (ANOVA) was used to evaluate the statistical significance of differences among the four drying groups. The post hoc Tukey's Honestly Significant Difference (HSD) test was applied to determine significant differences between groups. The level of statistical significance was set at $p<0.05$. All analyses were performed using IBM SPSS Statistics 27.0 software.

3 Results and Discussions

The findings obtained from the analyses reveal the critical importance of the choice of drying method in preserving the protein content and antioxidant activity of the crude whole-body larval extract (WBE) products of *Lucilia sericata* larvae. Preserving the quality of this complex bioactive mixture is imperative for maintaining its therapeutic potential in wound healing and antimicrobial applications.

3.1 Effect of Different Drying Methods on DPPH Radical Scavenging Activity

When comparing the DPPH radical scavenging activities of *Lucilia sericata* larvae extracts, significant differences were observed between the drying method and antioxidant capacity. The method with the lowest IC_{50} value is lyophilisation (freeze-drying) at 0.1350 mg/mL. This indicates that the extract prepared using the lyophilization method has a stronger free radical scavenging capacity compared to other methods.

Table 1. DPPH radical scavenging activity and antioxidant activity values of *L. sericata* larval extracts according to drying methods. Values are expressed as mean ± standard deviation.

***L. sericata* Larva ES Extracts**	**IC_{50} (mg/mL)** **IC_{50} (mg/mL)**	**Standard Deviation**
INFRARED	0,2546	0,0341
LYOPHILISATION	0,1350	0,0242
MICROWAVE	0,5721	0,0481
OVEN	0,3037	0,0210
Trolox (Positive control)	0,0023	0,0182
Each experiment was carried out with at least three replicates.		

One-way ANOVA of IC_{50} values showed significant differences among drying methods ($p < 0.05$); Tukey's post hoc test confirmed that Trolox had significantly superior antioxidant capacity compared to all extracts, and the lyophilisation drying method showed significantly higher antioxidant capacity than other methods.

When comparing the DPPH radical scavenging activities of *Lucilia sericata* larval extracts, significant differences were observed between the drying method and antioxidant capacity. The method with the lowest IC_{50} value was lyophilisation, at 0.1350 mg/mL. This indicates that the extract prepared using the lyophilisation method possesses a stronger free radical scavenging capacity compared to the other methods [Table 1]. The fact that the microwave extract has the highest IC_{50} value (0.5721 mg/mL) suggests that this method may degrade antioxidant components to a greater extent or that the extraction yield for these compounds may be lower. Although the IC_{50} value of the extract obtained by the oven method (0.3037 mg/mL) is close to that of the infrared extract (0.2546 mg/mL), it is not as effective as the infrared method. This result indicates that volatile or heat-sensitive antioxidant compounds are well preserved in the Infrared method as well, but that the lyophilisation method provides a more selective and effective extraction [Table 1]. The larval extract obtained by lyophilisation exhibited the highest radical scavenging activity, showing a lower IC_{50} (0.1350 mg/mL) in the DPPH assay compared to other drying methods; however, all extracts exhibited significantly lower activity compared to Trolox, the pure antioxidant reference (IC_{50} = 0.0023 mg/mL) [Table 1]. This can be explained by the relatively lower concentrations of active compounds in the extracts compared to Trolox and the presence of compound mixture effects.

3.2 Effect of Different Drying Methods on FRAP Activity

FRAP analysis was performed to determine the effect of different drying methods on the antioxidant capacity of the larval extract, and the results are presented in Table 2. The results indicate that the drying methods applied produced significant

differences in antioxidant capacity. The highest FRAP value was determined to be 45.44 in samples dried using the lyophilisation method. This was followed, in order, by the infrared drying method (40.16), the oven drying method (26.96) and the microwave drying method (23.37).

Table 2. Comparison of total antioxidant capacity (FRAP) in *Lucilia sericata* specimens following different drying methods

***L. sericata* Larva ES Extracts**	**mgTE/g**	**Standard Deviation**
LYOPHILISATION	45,4430	0,0037
INFRARED	40,1603	0,0082
OVEN	26,9620	0,0013
MICROWAVE	23,3755	0,0063

All data are presented as mean ± standard deviation (n = 3). Statistical analyses were performed using SPSS 27.0 software, and differences between groups were assessed using one-way analysis of variance (One-Way ANOVA). The Tukey post-hoc test was applied to identify significant differences, and a p-value of < 0.05 was considered statistically significant.

The results show that the freeze-drying process, carried out at low temperature and under vacuum, is more effective in preserving the antioxidant biomolecules present in the larval extract. Although the infrared method also provided a relatively high antioxidant capacity, it was not as effective as lyophilisation. In contrast, drying methods involving high temperatures, such as oven and microwave drying, may have caused thermal denaturation of proteins, enzymes and other biological molecules, leading to a reduction in antioxidant capacity [Table 2]. These results indicate that the drying method is a significant factor in the preservation of the biochemical components of the larval extract. It can be said that the freeze-drying method is a more suitable drying method, particularly in terms of preserving the stability of antioxidant compounds found in biological materials.

3.3 Effect of Different Drying Methods on Total Protein Content

Protein amounts measured by the Bradford method ranged from 44.3 to 237.1 mg BSA E/g depending on the drying method [Table 3]. These findings demonstrated that the drying process had a striking effect on protein solubility and stability. All analyses were performed in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Lyophilisation led the way with the highest protein yield, achieving a value of 237.1853 ± 0.0203 mg/ BSA eq/g dry weight [Table 3]. This was followed by infrared (173.0378 ± 0.0060 mg/ BSA eq/g dry weight), oven-dried (57.2964 ± 0.0128 mg/ BSA eq/g dry weight), and microwave-dried (44.3997 ± 0.0054 mg/ BSA eq/g dry weight) samples. The protein content in the microwave group was approximately 1/5 lower than that in the lyophilization group, and this difference was quite significant.

Table 3. Protein content of *L. sericata* larval extracts according to drying methods (Bradford methods assays). Values are presented as mean ± standard deviation.

	BSA (mg/g dry weight)	
***L. sericata* Larva ES Extracts**	**Total Protein Content**	**Standard Deviation**
LYOPHILISATION	237,1853	0,0203
INFRARED	173,0378	0,0060
OVEN	57,2964	0,0128
MICROWAVE	44,3997	0,0054

The effect of different drying methods on protein content in *Lucilia sericata* larva ES extracts specimens was evaluated using the Bradford method. Protein content was determined using a BSA standard and expressed in mg BSA E/g. **Values are presented as mean ± standard deviation (n = 3). Different letters indicate statistically significant differences between groups, as determined by one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).**

The results obtained with the Lowry method exhibited a distribution comparable to those of the Bradford assay, though consistently yielding lower values [Table 4]. Protein concentrations were ranked as follows: lyophilization (164,8125 ± 0,0056 mg/ BSA eq/g dry weight), infrared drying (144,4583 ± 0,0065 mg/ BSA eq/g dry weight), oven drying (81,1875 ± 0,0086 mg/ BSA eq/g dry weight), and microwave drying (57,1667± 0,0066 mg/ BSA eq/g dry weight). In both assays, statistically significant differences were observed between drying techniques and protein content ($p < 0.001$).

The effect of different drying methods on total protein content in *Lucilia sericata* specimens was evaluated using the Lowry method. Protein concentration was determined using a bovine serum albumin (BSA) standard curve and expressed in mg BSA E/g. **Values are presented as mean ± standard deviation (n = 3). Different letters indicate statistically significant differences**

Table 4. Protein content of *L. sericata* larval extracts according to drying methods (Lowry methods assays). Values are presented as mean ± standard deviation.

	BSA (mg/g dry weight)	
***L. sericata* Larva ES Extracts**	**Total Protein Content**	**Standard Deviation**
LYOPHILISATION	164,8125	0,0056
INFRARED	144,4583	0,0065
OVEN	81,1875	0,0086
MICROWAVE	57,1667	0,0066

between groups, as determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test ($p < 0.05$).

The present study provides a systematic evaluation of how four distinct drying methodologies—lyophilisation, infrared, microwave, and oven drying—impact the preservation of total protein content and antioxidant capacity in *Lucilia sericata* second instar (L2) larval extracts. Our findings confirm that the structural and functional integrity of these biotherapeutic products is highly sensitive to the intensity and duration of thermal stress. While previous studies have often been confirmatory, this research offers a critical comparative perspective that distinguishes the superior efficacy of non-thermal stabilization for pharmaceutical-grade larval extracts.

3.4 Superiority of Lyophilisation in Preserving Bioactive Integrity

The superiority of lyophilisation in preserving both antioxidant activity and protein content is consistent with its established role as the gold standard for stabilising thermolabile proteins and enzymes in pharmaceutical manufacturing[(10)],[(12)]. By operating under vacuum at sub-zero temperatures, sublimation significantly reduces hydrolytic and oxidative degradation rates[(11)],[(13)]. Our results align with Hoff et al. (2025)[(8)] and Keil et al. (2022)[(13)], who demonstrated lyophilisation as the most effective method for preserving antioxidant capacity in insect-derived materials. Lyoprotectant studies confirm that freeze-drying formulations maintaining glass-transition temperatures exhibit superior protein stability and reduced aggregation[(2)]. The approximately four-fold higher protein retention in our lyophilised group suggests preservation of essential proteolytic enzymes and AMPs[(5)],[(15)].

3.5 Critical Comparison of Thermal Drying Methods

A key advancement of this study is the critical comparison of infrared (IR) drying against traditional thermal methods. IR drying emerged as the second-best option, showing moderate preservation of bioactivity. This can be attributed to the penetrating nature of infrared radiation, which allows for more homogeneous and rapid heating compared to convective oven drying, thereby reducing the total duration of thermal exposure[(17)]. However, the significant reduction in protein content compared to lyophilisation suggests that even the moderate temperatures used in IR drying are sufficient to trigger partial denaturation of sensitive larval proteins[(24)]. In stark contrast, microwave and oven drying resulted in the lowest levels of bioactivity. The poor performance of microwave drying (IC50: 0.5721 mg/mL) is particularly noteworthy. While microwave drying is often praised for its speed, the dielectric heating mechanism can create localized "hotspots" that lead to rapid thermal degradation and the potential activation of degradative enzymes, which further depletes antioxidant capacity[(11)],[(13)]. This aligns with more recent critical evaluations noting that microwave energy can cause severe structural damage to insect proteins, reducing their solubility and functional capacity[(19)]. Oven drying at 60°C for 24 hours led to significant protein loss and reduced antioxidant capacity, likely due to prolonged exposure to hot air, which facilitates Maillard reactions and irreversible protein polymerization[(25)].

3.6 Mechanistic Insights: Oxidative Stress and Protein Stabilization

The parallel results of DPPH and FRAP assays provide a multifaceted perspective. Chronic wounds are characterised by extreme oxidative stress where excessive ROS inhibit cellular proliferation and promote tissue necrosis[(1)],[(2)]. The consistent superiority of lyophilised extracts in both assays indicates this method equally preserves compounds operating through distinct antioxidant mechanisms — radical scavenging and metal ion reducing power. The systematic difference between Bradford and Lowry protein measurements is consistent with the presence of non-proteinaceous bioactive molecules — including polysaccharides and phenolic compounds — that the Folin–Ciocalteu reagent also detects[(5)].

3.7 Implications for Standardized Larval Therapy

This study provides preliminary biochemical evidence that may support continued exploration of the transition from live larval therapy to standardised extract-based products; confirmation of comparable therapeutic efficacy will require further functional and clinical studies [8], [9]. Live larvae offer dynamic debridement but patient aversion and logistical challenges remain significant barriers [7]. Morris et al. (2023) confirmed potent antibiofilm properties of larval ES relevant to chronic wound management [15], while Mumford and Nigam (2024) identified standardised extract formats as a key facilitator for broader LT uptake [7].The industrial and economic aspects also merit consideration: while lyophilisation carries higher equipment and energy costs, infrared drying represents a viable lower-cost alternative for large-scale production [25].

3.8 The Industrial and Economic Aspects of Selecting a Drying Method

The selection of the drying method is important not only in terms of biological activity but also in terms of industrial applicability and cost. Although lyophilization is the method that preserves the highest bioactivity, it is expensive due to equipment costs and energy consumption. Infrared and oven drying are more economical options, but they preserve the biological potential of the extract to a more limited extent. Therefore, a cost-effectiveness balance must be considered in industrial-scale production: based on the present biochemical findings, lyophilisation may be preferable when preservation of protein content and antioxidant activity is prioritised, while infrared or oven drying may represent more suitable alternatives for large-scale production, offering a reasonable level of antioxidant activity at lower cost; confirmation of any resulting difference in therapeutic performance would require further functional assays.

3.9 Limitations and Future Directions

A further methodological limitation concerns the nature of replication used for the biochemical assays: the triplicate measurements reported for each drying method represent technical replicates – repeated measurements performed on a single prepared larval extract – rather than biological replicates derived from independently reared, dried, and processed larval batches. While reporting DPPH, ABTS, or FRAP assays in triplicate from a single extract is common practice in the antioxidant-assay literature Shah & Modi and Rumpf et al., [26], [27]such a design is informative primarily about the reproducibility of the measurement technique rather than about true biological variability between batches [28]. Consequently, the very low standard deviations observed in the present dataset should be interpreted as indices of assay precision rather than as estimates of biological variance, and the statistical comparisons among drying methods should be regarded as preliminary. Future studies incorporating independent biological replicates – separate larval cohorts reared and processed under identical conditions – are recommended to confirm the robustness of the differences observed among drying methods. In addition, formal tests of normality (Shapiro-Wilk) and homogeneity of variance (Levene's test) were not performed prior to one-way ANOVA in this study. Given the small number of replicates per group (n = 3, technical replicates as noted above), such tests would have had limited statistical power to detect violations of these assumptions and are of limited practical value as a screening step at this sample size; one-way ANOVA is nonetheless comparatively robust to moderate deviations from normality under the balanced (equal n) design used here. The absence of formal assumption testing is acknowledged as a further limitation of the statistical analysis, and it is recommended that future studies employing larger numbers of independent biological replicates formally test and report these assumptions. Furthermore, although an equal starting quantity of fresh L2 larvae was dried to a constant-weight endpoint under each of the four drying protocols, the final dry weight, residual moisture content, and water activity of the resulting powders were not quantitatively measured in this study. Reaching a constant-weight endpoint does not necessarily indicate equivalent residual moisture across drying technologies, since rapid, surface-heating methods such as microwave and infrared drying can promote surface case-hardening, whereby a dried outer layer slows further moisture loss and registers as "constant weight" while retained internal moisture remains higher than in methods such as lyophilisation, which removes water via sublimation. Because downstream analyses were performed on a fixed mass (10 mg) of each dried powder dissolved in a fixed volume (10 mL), unmeasured differences in residual moisture between groups cannot be excluded as a contributing factor to the observed differences in protein and antioxidant values across drying methods. Future studies should quantify final moisture content (e.g., by loss-on-drying or Karl Fischer titration), water activity (aw meter), and drying/recovery yield (final dry weight / initial fresh weight $\times$ 100) for each drying method to enable moisture-corrected comparisons. On the other hand a limitation of the current study is the focus on total protein and antioxidant capacity rather than the activity of specific enzymes. Future research should utilize SDS-PAGE to characterize the specific protein profiles across different drying regimes [3], [5]. Additionally, evaluating the antimicrobial efficacy of the extracts against specific clinical isolates like MRSA after different drying processes would provide direct evidence of their therapeutic stability [11]. Investigating the impact of these drying methods on the larval lipid profile would also be valuable for developing complete larval-based formulations [22].

4 Conclusion

This study underscores that the selection of the drying method is a critical determinant in the conversion of *Lucilia sericata* larvae into standardized, high-quality pharmaceutical or nutraceutical products. Our comprehensive comparative analysis demonstrates unequivocally that lyophilisation (freeze-drying) is the superior method for preserving the delicate biotherapeutic potential of the larvae. Specifically, lyophilisation retained up to four times more protein content and exhibited significantly higher antioxidant capacity compared to conventional thermal methods [8], [13]. The core contribution of this study lies in providing robust, quantitative evidence that the duration and intensity of thermal stress during processing directly degrade the heat-sensitive proteins and antioxidant molecules essential for the efficacy of Larval Therapy (LT) [10], [12]. By establishing lyophilisation as the superior drying method with respect to protein and antioxidant preservation, this research offers a preliminary, evidence-based foundation for developing a standardised protocol for the industrial-scale production of stable, bioactive larval extracts. Ultimately, the findings of this study address the critical bottleneck of biomolecule degradation during extract production, paving the way for the development of standardized, storable, and patient-friendly maggot-derived therapies.

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