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COMPARISON [2] OF DIFFERENT DRYING METHODS ON THE PHYSICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF *Rosmarinus officinalis* L.

Abstract

[5] Rosemary (*Rosmarinus officinalis* L.) is an aromatic plant widely valued for its antioxidant properties, yet the impact of different drying methods on its bioactive potential remains underexplored in tropical contexts. This study evaluated the effect of three drying methods—sun drying, solar dryer drying, and microwave-vacuum drying—on the physical, antioxidant, and microbiological properties of rosemary leaves harvested in the Dominican Republic. Microwave-vacuum drying drastically reduced processing time (5 min) compared to sun drying (20 h) and solar dryer drying (12 h). This method best preserved the original color (L, a, b* values closest to fresh rosemary), achieved the lowest water activity (0.35), and exhibited [2] the highest retention of antioxidant capacity (IC_{50} = 25.7 µg/mL) and vitamin C content (0.03%). Furthermore, microwave-vacuum drying yielded the lowest counts of aerobic mesophiles (2.0×10^3 CFU/g) and molds/yeasts (1.0×10^2 CFU/g), suggesting an additional sterilizing effect. These findings demonstrate that microwave-vacuum drying is the most efficient and qualitatively superior method for rosemary preservation under tropical conditions, combining rapid processing, microbiological stability, and optimal retention [1] of bioactive compounds. This technology represents a promising alternative for value-added processing of aromatic herbs in tropical regions.

Keywords: Rosemary, Microwave drying, Vacuum drying, Antioxidant activity, Water activity, Postharvest technology, Tropical agriculture, Dominican Republic

1.Introduction:-

Drying is a fundamental unit operation for food preservation and subsequent industrial processing. This process involves the removal of unbound water during the constant-rate period, followed by the elimination of internal moisture. While evaporation initially occurs at the surface, the removal of bound water is essential to obtain a shelf stable, microbiologically safe product [1]. The reduction of moisture content inhibits bacterial growth and proliferation, thereby extending product shelf life. Additionally, drying affects enzymatic activity, sensory properties, and microbial development [2]. Microbial stability is generally achieved when water activity (aw) falls below 0.6. Among emerging dehydration technologies, microwave-vacuum drying (MVD) has gained considerable attention for overcoming the limitations of conventional drying methods while improving the quality of dehydrated products [3, 4]. The most frequently employed microwave frequencies for food drying are 915 MHz and 2450 MHz. This technology integrates four essential requirements for industrial drying: high operational speed, energy efficiency, low operating costs, and high product quality. The vacuum environment ensures rapid mass transfer at low temperatures, while microwave heating accelerates energy transfer and reduces energy consumption by approximately 50% compared to conventional systems [5]. Furthermore, the absence of air during processing prevents oxidative degradation. MVD has been successfully applied to various food matrices, including fruits, vegetables, and aromatic herbs [6], with documented applications in apple, blackcurrant, blueberry, pomegranate, garlic, strawberry, and tomato [7–10]. Recent advances have focused on hybrid approaches combining microwave drying with complementary technologies, yielding excellent results [11–14]. Rosemary (Salvia Rosmarinus Spenn., syn. Rosmarinus officinalis L.), a member of the Lamiaceae family, is a perennial aromatic shrub native to the Mediterranean region, now cultivated worldwide for its aromatic, ornamental, and medicinal properties [15–17]. Rosemary is widely recognized as one of the spices with the highest antioxidant activity [18]. Its essential oil also exhibits antibacterial, antifungal, and anticancer properties. Aromatic plants are typically dried prior to extraction to reduce moisture content. The drying method employed significantly influences both the content and composition of essential oils [19–22].

Several studies have examined the effect of drying methods on rosemary's functional properties and quality [23], employing techniques such as sun drying, shade drying, oven drying, and microwave drying. According to Melese et al. [24], fresh or shade-dried rosemary yields the highest essential oil extraction efficiency. The Dominican Republic possesses favorable agroecological conditions for rosemary cultivation; however, limited research exists on optimizing postharvest processing technologies adapted to tropical conditions. This knowledge gap constrains the development of value-added products and limits market opportunities for local producers. Therefore, this study presents a comparative evaluation of three drying methods for rosemary—sun drying, solar dryer drying, and microwave-vacuum drying—by assessing drying kinetics, water activity, color parameters, antioxidant activity, vitamin C retention, and microbiological quality. The objective is to identify the most suitable drying method for preserving the functional properties of rosemary under tropical conditions, thereby contributing to the technological modernization of the aromatic herbs value chain in the Dominican Republic.

2.Materials and Methods:-

2.1. Plant material

Fresh [1] rosemary (*Rosmarinus officinalis* L.) leaves (5 kg) at vegetative stage, uniform and free from visible damage, were collected from a two-year-old plantation at Agroecológica Iguazú farm, located in Camino a Manabao, Jarabacoa, La Vega Province, Dominican Republic (19°07′ N, 70°38′ W). Harvesting was conducted manually during the morning hours (07:00–09:00) in July 2024. Leaves were immediately transported to the laboratory under refrigerated conditions (4 ± 1°C) and processed within 24 h.

2.2. Drying treatments

2.2.1. Sun drying

Fresh rosemary leaves (500 ± 5 g) were uniformly distributed on perforated stainless-steel trays (90 × 30 × 10 cm, 2.5 mm perforations). Trays were placed outdoors under direct sunlight for 20 h (08:00–16:00, followed by overnight exposure). Solar intensity was monitored using a Fluke FLK-IRR1-SOL pyranometer (Fluke Corporation, Everett, WA, USA) (0.80–1.00 kW/m²). Ambient relative humidity ranged from 70% to 80%. Tray temperature varied between 35–40°C during daytime and 24–26°C overnight.

2.2.2. Solar dryer drying

A closed-type solar dryer (Wuhan Acme Agro Tech Co., Ltd., Wuhan, China) measuring 6.0 × 3.5 m with a 2.0 m arch, translucent polycarbonate UV-filtering cover, temperature control system, and solar-powered air extractors was employed. The same tray type and sample quantity (500 ± 5 g) as in sun drying were used. Temperature was maintained below 50°C. Moisture content was determined every 2 h using a Sartorius MA 160 moisture analyzer (Sartorius AG, Göttingen, Germany) until a final moisture content <12% (wet basis) was achieved.

2.2.3. Microwave-Vacuum drying

A microwave-vacuum dehydration and sterilization system (Shandong Dongxuja DXY-16ZK, Shandong, China) with 16–20 kWh power consumption, 15 kg/h capacity, and vacuum range of 0.08–0.095 MPa was employed. Fresh rosemary leaves (500 ± 5 g) were uniformly distributed on two trays. Operating conditions were: vacuum pressure 0.8–1.0 kPa, microwave frequency 915 MHz, and temperature 40–45°C. Drying was performed for 2, 4, and 6 min intervals. Final moisture content (<12% wet basis) was verified using the Sartorius MA 160 moisture analyzer.

2.3. Analytical determinations

2.3.1. Moisture content

Moisture content was determined gravimetrically using a Sartorius MA 160 infrared moisture analyzer (Sartorius AG, Göttingen, Germany) at 105°C until constant weight. Results were expressed as percentage wet basis. All measurements were performed in quintuplicate.

2.3.2. Water activity (aw)

Water activity was measured at 25 ± 3°C using an HD-3A water activity meter (Hangzhou West Tune Trading Co., Ltd., Hangzhou, China). Samples were cut into approximately 0.5 cm particles prior to analysis. Measurements were performed in triplicate.

2.3.3. Color analysis

Color parameters were determined using a Konica-Minolta CR-20 portable colorimeter (Konica Minolta, Tokyo, Japan) calibrated with a standard white plate. Measurements were expressed in the CIE L*a*b* color space, where L* denotes lightness (0 = black, 100 = white), a* indicates redness (+) to greenness (−), and b* indicates yellowness (+) to blueness (−). Ten replicate measurements were performed per treatment.

2.3.4. Antioxidant activity (DPPH Assay)

Antioxidant capacity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method [25, 26]. Rosemary extracts were prepared by macerating 1 g of dried sample in 10 mL of methanol (80% v/v) for 24 h at room temperature in darkness, followed by filtration (Whatman No. 1). Serial dilutions were prepared (5–200 µg/mL). An aliquot (0.1 mL) of each dilution was mixed with 3.9 mL of

DPPH methanolic solution (0.1 mM). The mixture was incubated in darkness for 30 min at room temperature. Absorbance was measured at 515 nm using a Thermo Scientific Genesys 10 UV-Visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Methanol was used as blank. DPPH solution without extract served as control. The percentage of inhibition was calculated as:

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The IC_{50} value (concentration required to scavenge 50% of DPPH radicals) was calculated by interpolation from the linear regression of inhibition percentage versus concentration. All analyses were performed in triplicate.

2.3.5. Ascorbic acid determination

Ascorbic acid content was quantified by high-performance liquid chromatography (HPLC) using an YL9100 system (Young Lin, Anyang, Korea) equipped with a quaternary pump, autosampler, and UV-Vis detector. Separation was achieved on a PhenoSphere-Next C18 column (250 × 4.6 mm, 5 μm particle size) maintained at 30°C. The mobile phase consisted of 0.1% phosphoric acid in water (pH 2.5) at a flow rate of 1.0 mL/min. Injection volume was 20 μL. Detection was performed at 278 nm. Quantification was accomplished using an external calibration curve constructed with authentic L-ascorbic acid standard (Sigma-Aldrich, St. Louis, MO, USA) at concentrations ranging from 5 to 100 μg/mL. Results were expressed as percentage (g ascorbic acid/100 g dry weight). Analyses were performed in triplicate.

2.3.6. Microbiological analysis

Aerobic mesophilic bacteria were enumerated according to ISO 4833-1:2013/Amd 1:2022. Briefly, 10 g of sample were homogenized with 90 mL of sterile peptone water (0.1% w/v) in a Stomacher blender for 2 min. Serial decimal dilutions were prepared, and 1 mL aliquots were plated in duplicate on plate count agar (PCA). Plates were incubated at 30 ± 1°C for 72 ± 3 h. Molds and yeasts were enumerated according to ISO 21527-1:2008. Dichloran rose-bengal chloramphenicol (DRBC) agar was used, with incubation at 25 ± 1°C for 5–7 days. Results were expressed as colony-forming units per gram (CFU/g). All analyses were performed in duplicate.

2.4. Statistical analysis

All determinations were performed with five replicates for drying time, moisture content, water activity, and color, and three replicates for antioxidant activity, vitamin C, and microbiological analyses. Results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test was performed to identify significant differences among treatments. Statistical

significance was set at $p < 0.05$. All statistical analyses were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion:-

3.1. Drying kinetics and moisture content

Table 1 presents the drying times, final moisture contents, water activity values, and color parameters of rosemary samples subjected to the three drying treatments compared to fresh rosemary. Microwave-vacuum drying dramatically reduced the time required to achieve a final moisture content below 12% (wet basis) compared to conventional methods. Complete drying was achieved in only 5 min with MVD, whereas solar dryer drying required 12 h and sun drying required 20 h. This represents a 99.3% and 99.6% reduction in processing time compared to solar dryer and sun drying, respectively. These results are consistent with previous studies reporting the superior drying efficiency of microwave-vacuum technology for herbs and spices [8, 27]. The rapid drying rate observed with MVD [2] can be attributed to the combined effects of volumetric heating via microwave radiation and the reduced boiling point of water under vacuum conditions, which creates a large pressure gradient between the interior and surface of the plant material, thereby accelerating moisture migration [5].

All drying treatments successfully reduced the initial moisture content of fresh rosemary (67.3%) to levels below 12%, meeting the recommended moisture content for shelf-stable dried herbs (<12%). The lowest final moisture content (10.0%) was achieved with MVD, followed by solar dryer drying (11.1%) and sun drying (11.6%). Although these differences were statistically significant ($p < 0.05$), all values are within acceptable limits for microbiological stability and storage.

Table 1: Effect of drying method on drying time, moisture content, water

activity, and color parameters of 161 rosemary (Rosmarinus officinalis L.). 162 Parameter Fresh rosemary Sun drying Solar dryer drying Microwave-vacuum drying Drying time — 20.0 ± 1.4 hour a 12.0 ± 0.8 hour b 5.0 ± 0.2 min c Moisture content (%) 67.3 ± 0.5 a 11.6 ± 0.3 b 11.1 ± 0.4 b 10.0 ± 0.2 c Water activity (aw) 0.90 ± 0.05 a 0.50 ± 0.08 b 0.45 ± 0.04 bc 0.35 ± 0.02 c Color (L* a* b*) 35.2 ± 1.1, -7.1 ± 0.3, 16.3 ± 0.5 48.5 ± 1.8, -0.8 ± 0.1, 22.4 ± 0.7 44.3 ± 1.5, -2.1 ± 0.2, 17.2 ± 0.6 38.1 ± 1.2, -6.2 ± 0.3, 17.0 ± 0.5 Values represent mean ± standard deviation. Different letters within the same row indicate significant differences (p 163 < 0.05) according to Tukey's HSD test. 164 3.2. Water activity 165 Water activity is a critical parameter for predicting the microbiological stability and shelf life of dried products. 166 Fresh rosemary exhibited a high aw value (0.90), characteristic of fresh plant tissues and conducive to rapid 167 microbial proliferation. All drying treatments significantly reduced aw values (p < 0.05), with MVD achieving the 168 lowest aw (0.35), followed by solar dryer drying (0.45) and sun drying (0.50). These values are all below the critical 169 threshold of 0.60 required for the growth of most spoilage microorganisms, including bacteria, yeasts, and molds 170 [28]. The exceptionally low aw achieved with MVD (0.35) provides a substantial safety margin and indicates 171 superior long-term storage stability. This finding aligns with Calín-Sánchez et al. [8], who reported aw values of 172 0.32–0.38 for microwave-vacuum dried pomegranate arils. The lower aw achieved with MVD can be attributed to 173 the more efficient removal of strongly bound water under vacuum conditions. 174 175 3.3. Color parameters 176 Color is a primary quality attribute influencing consumer acceptance and market value of dried herbs. The color 177 parameters of fresh and dried rosemary samples are presented in Table 1. Fresh rosemary exhibited characteristic 178

dark green coloration with low L* (35.2), negative a* (−7.1), and moderately positive b* (16.3). Sun drying resulted 179 in the most pronounced color alteration, with significantly increased lightness (L* = 48.5), shift toward red tones (a* 180 = −0.8), and increased yellowness (b* = 22.4). This extensive color degradation is attributable to prolonged 181 exposure to solar UV radiation and oxidative conditions, which promote chlorophyll degradation and the formation 182 of pheophytins [29]. Solar dryer drying, incorporating UV-filtering polycarbonate and reduced drying time, yielded 183 intermediate color preservation (L^* = 44.3, a^* = −2.1, b^* = 17.2). 184 185 Microwave-vacuum drying demonstrated superior color preservation, with L, a, and b* values (38.1, −6.2, and 17.0, 186 respectively) closest to those of fresh rosemary. The minimal color change observed with MVD can be explained by 187 three factors: (1) the extremely short drying time limits the duration of thermal exposure; (2) the low-temperature 188 environment (40–45°C) reduces the rate of chlorophyll degradation; and (3) the oxygen-deficient vacuum 189 atmosphere minimizes oxidative reactions [23, 29]. These results confirm the effectiveness of MVD in preserving 190 the natural green color of rosemary, which is highly desirable for both culinary and nutraceutical applications. 191 3.4. 2 Antioxidant activity and vitamin C retention 192 Table 2 presents the effects of different drying methods on the antioxidant capacity (IC_{50}), vitamin C content, and 193 microbiological quality of rosemary. Fresh rosemary exhibited strong antioxidant activity, with an IC_{50} value of 18.3 194 µg/mL, consistent with previously reported values for this species [18]. All drying treatments resulted in some loss 195 of antioxidant capacity, as evidenced by increased IC_{50} values. However, 2 the extent of this loss varied considerably 196 among methods. 197 Sun drying caused the most severe degradation of antioxidant compounds, with IC_{50} increasing more than 8 -fold 198 (148.2 µg/mL) compared to fresh rosemary. This dramatic loss is attributable to prolonged exposure to heat, light, 199 and oxygen, which promote the oxidative degradation of phenolic compounds and other antioxidant metabolites [30]. 200 Solar dryer drying, while less detrimental than sun drying, still resulted in substantial antioxidant loss (IC_{50} = 87.9 201 µg/mL). 202 Remarkably, microwave-vacuum drying preserved antioxidant capacity to a much greater extent, with IC_{50} values 203 (25.7 µg/mL) only 40% higher than fresh rosemary and significantly lower than both conventional drying methods 204 ($p < 0.05$). This represents approximately 86% retention of the original 4 antioxidant activity, compared to only 41% 205 for solar dryer drying and 12% for sun drying. These findings corroborate those ofGarcía et al. [30], who reported superior retention of phenolic compounds and antioxidant activity in microwave-vacuum dried herbs compared to conventionally dried samples. Table 2: Effect of drying method on antioxidant capacity, vitamin C content, and microbiological counts of rosemary (Rosmarinus officinalis L.). Parameter Fresh rosemary Sun drying Solar dryer drying Microwave-vacuum drying IC_{50} (µg/mL) 18.3 ± 1.2 a 148.2 ± 8.7 d 87.9 ± 5.3 c 25.7 ± 1.8 b Vitamin C (%) 0.04 ± 0.005 a ND 0.01 ± 0.002 c 0.03 ± 0.003 b Aerobic mesophiles (CFU/g) $2.0 \times 10^5 \pm 1.2 \times 10^4$ a $3.8 \times 10^4 \pm 2.1 \times 10^3$ b $1.1 \times 10^4 \pm 8.2 \times 10^2$ c $2.0 \times 10^3 \pm 1.5 \times 10^2$ d Molds and yeasts (CFU/g) $3.2 \times 10^4 \pm 2.4 \times 10^3$ a $1.5 \times 10^3 \pm 1.1 \times 10^2$ b $6.5 \times 10^2 \pm 4.8 \times 10^1$ c $1.0 \times 10^2 \pm 0.8 \times 10^1$ d Values represent mean ± standard deviation. Different letters within the same row indicate significant differences ($p < 0.05$) according to Tukey's HSD test. ND: not detected. A similar trend was observed for vitamin C, a thermolabile micronutrient highly susceptible to oxidative degradation. Fresh rosemary contained 0.04% vitamin C (dry weight basis). After sun drying, vitamin C was completely undetectable, while solar dryer drying retained only 25% of the original content (0.01%). In contrast, MVD retained 75% of the initial vitamin C content (0.03%), representing a statistically significant improvement ($p < 0.05$). The superior retention of both antioxidant capacity and vitamin C with MVD can be attributed to three mechanisms: (1)

rapid moisture removal minimizes the time available for thermally-induced degradation reactions; (2) low processing temperatures reduce the kinetic energy available for degradation pathways; and (3) the oxygen-depleted vacuum environment limits oxidative reactions [9, 10]. 3.5. Microbiological quality Fresh rosemary exhibited substantial microbial loads, with aerobic mesophile counts of 2.0×10^5 CFU/g and mold/yeast counts of 3.2×10^4 CFU/g (Table 2). These values are typical for fresh aromatic herbs and reflect the natural epiphytic microbiota as well as potential contamination from soil and handling [28]. All drying treatments significantly reduced microbial counts ($p < 0.05$). Sun drying reduced aerobic mesophiles by approximately 1.7 log cycles (3.8×10^4 CFU/g) and molds/yeasts by 1.3 log cycles (1.5×10^3 CFU/g). Solar dryer drying achieved greater reductions: 2.3 log cycles for aerobic mesophiles (1.1×10^4 CFU/g) and 1.7 log cycles for molds/yeasts (6.5×10^2 CFU/g). These reductions are primarily attributable to the decreased water activity, which creates an inhospitable environment for microbial proliferation. Microwave-vacuum drying achieved the most substantial microbial reduction, decreasing aerobic mesophile counts by 2.0 log cycles (2.0×10^3 CFU/g) and mold/yeast counts by 2.5 log cycles (1.0×10^2 CFU/g) compared to sun dried samples. The final microbial loads achieved with MVD are well below the international microbiological limits for dried herbs (typically $\leq 10^4$–10^5 CFU/g for aerobic mesophiles) [31]. The enhanced microbial inactivation observed with MVD cannot be explained solely by water activity reduction, as the a_w difference between MVD (0.35) and solar dryer drying (0.45) is relatively modest. This suggests an additional non-thermal microbial inactivation mechanism, likely related to the electromagnetic effects of microwave radiation. Microwave exposure can cause microbial cell death through electroporation of cell membranes, disruption of protein and nucleic acid synthesis, and localized thermal effects at the cellular level [22]. The combination of vacuum conditions with microwave radiation appears to synergistically enhance microbial inactivation while preserving product quality.

3.6. Implications for tropical herb processing

The findings of this study have significant implications for the development of value-added processing chains for aromatic herbs in tropical regions such as the Dominican Republic. Traditional sun drying, despite its low capital cost, presents multiple disadvantages under tropical conditions: (1) high ambient humidity prolongs drying time and increases the risk of microbial spoilage; (2) intense solar radiation causes extensive color degradation and loss of bioactive compounds; (3) exposure to environmental contaminants (dust, insects, birds) compromises food safety; and (4) dependence on weather conditions limits process control and reproducibility. Solar dryer drying represents an intermediate technological solution, offering improved control over drying conditions and moderate product quality, but still requiring prolonged processing times (12 h) and resulting in substantial loss of bioactive compounds. Microwave-vacuum drying, while requiring higher capital investment, offers compelling advantages for premium herb processing: (1) drastic reduction in processing time (from hours to minutes) enables just-in-time processing and reduces inventory requirements; (2) superior preservation of color, [2] antioxidant activity, and vitamin C content commands premium prices in high-value markets; (3) enhanced microbiological reduction may eliminate the need for additional decontamination steps such as ethylene oxide treatment or irradiation; and (4) the closed-system design eliminates contamination risks and enables reproducible, weather-independent processing. For small and medium-scale producers in tropical countries, shared ownership models or centralized processing service centers could make MVD technology economically accessible. Further research should include comprehensive techno-economic analysis and life cycle assessment to evaluate the feasibility of MVD adoption under various production scales. 4. Conclusions:- This study demonstrates that the drying method employed profoundly affects the physical, chemical, and microbiological quality of [1] rosemary (Rosmarinus officinalis L.). The following conclusions can be drawn:

1. Microwave-vacuum drying demonstrated exceptional efficiency, reducing processing time to 5 min—a 99.3% and 99.6% reduction compared to solar dryer drying (12 h) and sun drying (20 h), respectively. 2. MVD best preserved the original color characteristics of rosemary, with L, a, and b* values most closely approximating fresh rosemary. This method also achieved the lowest water activity (0.35), ensuring superior long-term microbiological stability. 3. MVD exhibited superior preservation of functional properties, retaining approximately 86% of the original antioxidant capacity (IC_{50} = 25.7 μg/mL) and 75% of vitamin C content. In contrast, sun drying resulted in nearly complete loss of antioxidant activity and undetectable vitamin C levels. 4. MVD

achieved the most substantial microbial reduction, yielding final counts of 2.0×10^3 CFU/g for aerobic mesophiles and 1.0×10^2 CFU/g for molds/yeasts. The enhanced microbial inactivation observed with MVD suggests an additional non-thermal sterilizing effect of microwave radiation under vacuum conditions.

5. Based on the comprehensive evaluation of drying efficiency, product quality, and microbiological safety, microwave-vacuum drying is recommended as the optimal method for rosemary dehydration under tropical conditions. This technology offers a viable pathway for upgrading traditional herb processing chains, enabling Dominican producers to access high-value markets for premium-quality dried herbs. Future research should focus on: (1) optimizing MVD parameters (microwave power, vacuum level, intermittent cycling) for different aromatic species; (2) evaluating the stability of MVD-dried rosemary during long-term storage under tropical conditions; (3) conducting comprehensive life cycle assessment and economic feasibility studies; and (4) scaling up the technology for industrial implementation. Additionally, the potential application of MVD for other high-value tropical herbs and spices cultivated in the Dominican Republic (e.g., oregano, lemongrass, citronella) warrants investigation.

5. Data availability:- The data used to support the findings of this study are available from the corresponding author upon reasonable request.

6. Conflicts of interest:- The authors declare that there are no conflicts of interest regarding the publication of this article.

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