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Assessment of a *Mangifera indica* seed extract in topical cream as an adjuvant treatment on surgical site infections

Abstract

The high incidence of surgical site infections (SSI) is a serious problem in developing countries and has an impact on public health. Therefore, it was proposed to evaluate the antimicrobial activity of different topical formulations based on M. indica seed extract, both in vitro and on surgical site infections of patients admitted to the Hospital Central de Maracay (Aragua-Venezuela). Different topical formulations were evaluated in vitro through various assays. Subsequently, patients diagnosed with surgical site infection were selected and divided into two groups: the first (A) received treatment with the topical extract-based formulation, and a second (B) received a placebo. Both groups received their respective antibiotic therapy. All concentrations showed in vitro antimicrobial activity in both certified and wild strains. In patients, Group A showed an earlier resolution of wound inflammation and discharge with 80% of patients having no signs of inflammation (phlogosis) by day 6 ($p=0.0003$). Wound healing was faster in the experimental group, with 80% granulation by day 6 and 100% re-epithelialization by day 10, compared to 50% in the control group ($p<0.005$). The healing time was shorter in the experimental group (6.0±0.9 days) compared to the control one (7.6±1.1 days). The cream formulated with 15% mango seed extract proved to be an efficient adjuvant for the treatment of patients with this type of infection; since it improved the times for granulation, re-epithelialization, and overall wound healing.

Keywords: mango seed, postoperative infections, natural products, re-epithelialization, wound healing, antimicrobial

Volume 11 Issue 1 - 2026

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Received: August 24, 2026 | **Published:** September 25, 2026

Introduction

Surgical Site Infections (SSI) are common complications with a prevalence of 1% to 3% in patients undergoing surgery within 30 days after the intervention.[1–2] In low and middle-income countries, this percentage is higher than 10%. In Africa it has been reported that 20% of women who undergo cesarean section surgery contract a surgical site infection.[3] SSIs are caused by microorganisms generally resistant to antimicrobials, which penetrate the skin through incisions made during hospital surgery and increase postoperative morbidity and mortality rates.[2] Depending on the surgical site and the type of intervention, patients with an SSI have a 2 to 11 times higher risk of death than patients without SSI. Furthermore; 77% of deaths in patients with SSI are directly related to the infection.[1] The costs ascribable to SSI vary according to the type of surgical procedure, medical implants, and the type of infectious pathogen, but in general, the medical expenses are estimated to increase between 1.4 to 3 times more per patient. In the United States; SSIs account for between $3.5 to $10 billion annually in healthcare costs[1] and contribute to a significant raise of the inpatient days in hospitals.[3]

In Venezuela, there are no official statistics on the prevalence of this type of infection. However, studies conducted in public hospitals have indicated values between 4.7% and 9.7%. Piñango et al.[4] in their research conducted at the Miguel Pérez Carreño Hospital in Caracas between January 2019 and July 2021 with 1341 patients, found that the overall incidence of SSI was 9.77%. The majority of infections occurred in patients undergoing emergency surgeries (10.7%). The pathologies with the highest percentage of infection were abdominal trauma, followed by intestinal obstruction. The most frequently isolated microorganism was *Escherichia coli*. Fernández et al.[5] in a retrospective study conducted at Hospital Universitario de Caracas in 2019; based on the medical records of 48 patients; found a prevalence of 4.69% (95% CI = 2.79 - 7.16%). As in the research done by Piñango et al.,[4] the majority occurred after emergency surgeries (87.50%) specifically appendectomies (45.83%) and cholecystectomies (10.41%). Among the microorganisms identified, *E. coli* (10.50%), *Enterococcus* sp. and *Klebsiella pneumoniae* (6.24%), *Morganella morganii* (2.1%) and *Streptococcus pneumoniae* (2.1%) showed higher prevalence.

To lessen the incidence of SSIs; hospital centers regularly consider strategies and protocols to manage the different risk factors; ranging from aspects related to the patient, the procedure, the skills of the surgical team and the hospital environment.[1,5] When the patient has SSI complications, a multidisciplinary team assessment is required in the first instance; which must include the surgeon, an infectious disease physician, and a microbiologist in order to design the

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therapeutic plan that includes both local wound management such as drainage, cure, use of special creams and dressings, etc., as well as the comprehensive antibiotic therapy that will be applied. The appropriate use of antimicrobials is a key point, comprising the initial selection according to the pathology and the correct dose, which defines the subsequent evolution. Individual and institutional risks to certain germs or resistance mechanisms must also be considered.[6]

Antibiotic treatment for SSIs generally begins with de-escalation therapy, which consists of the use of broad-spectrum drugs, and subsequently, upon obtaining the bacteriological result and identifying the infectious agents the treatment is adjusted, reducing the spectrum of initial antimicrobial coverage in order to avoid the development of bacterial resistance.[6] However, the problems of antimicrobial resistance are increasingly frequent and compromise the success of treatments.[7,8] For this reason, various researchers are working on the formulation of topicals ointments or lotions based on natural products that can be used as adjuvants in the antibiotic therapy of SSIs in order to promote treatment success. Such as the case of Amgad et al.,[9] who developed ointments and creams based on mango seed extracts (*Mangifera indica*) and found their high potential as a topical antimicrobial agent. The authors attributed this property to its high concentration of polyphenolic compounds, especially mangiferin. For their part, authors such as Raju et al.[10] and Noguera et al.,[11] demonstrated the antibacterial effectiveness of mango seed extracts on different strains of *Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus*, including methicillin-resistant strains (MRSA).

The proper management of the infected wound is essential, and directly impacts the morbidity and mortality rates in patients. Therefore, the present investigation purpose is to evaluate the potential of topical products formulated with *M. indica* seed extract as adjuvants for the treatment of SSIs. The specific goals were to determine the *in vitro* antibacterial potential of the formulated topical products, and then describe their effect on a clinical level in patients with SSI.

Materials and methods

The study was experimental and developed in two phases: one at the laboratory with *in vitro* assays and another phase of a longitudinal, double-blind, controlled clinical trial; carried out between January and November 2024, and approved by the Bioethics Committee of the Hospital Central de Maracay (Nro CIBSADHCM0123).

In vitro phase

Biological material

Mango (*Mangifera indica*) seeds were used for the task and those were acquired in fruit stores in the city of Maracay, Girardot municipality of Aragua state.

Certified strains from the American Type Culture Collection (ATCC) and wild strains were used for the antibacterial activity tests. Work was done with *Escherichia coli* ATCC25922 and *Staphilococcus aureus* ATCC25923, obtained from the strain bank of the "Dr. Francisco Javier Triana Alonso" Biomedical Research Institute of the University of Carabobo (BIOMED-UC). Wild strains isolated from patients with SSI infection, which were identified by biochemical tests in the hospital's microbiology laboratory, were also used.

Preparation of the extract

The mangoes were pulped to extract the seeds, which were then washed and dried to constant weight. Then the pericarp and mesocarp were removed until reaching the endocarp, which was subjected to mechanical grinding in an Oster © blender. From this powder, the extraction was carried out according to the methodology proposed by Noguera et al.[11]

Formulation of the topical dressing

Two types of topical agents, ointment and cream, were prepared with the addition of mango seed extract at 10% and 15%. They were prepared in a compounding pharmacy laboratory to guarantee the evenness of the products, the ointment (cetoestearilic alcohol 5 %, lanoline 5 %, parafine 5 % and vaseline 85%) and cream (stearic acid 140 g, sodium hydroxide 30 g, glycerin 280 g and water 550 g). An ointment and a cream without extract (0%) were also formulated, with the addition of 15% ethanol, which were used as controls or placebo.[12]

Determination of antibacterial activity

The agar gel diffusion method was used to evaluate the effect of the ointment and cream at 0%, 10%, and 15% concentration on the growth of the certified strains. For this, the bacteria were grown in enriched culture medium under continuous agitation for 24 hours, to reactivate their metabolic functions, and then they were plated on Mueller-Hinton agar using the exhaustion technique. Filter paper discs impregnated with the ointment and cream with the corresponding extract percentages were placed on the cultured plates. The plates were incubated at 37 °C and growth halos were measured in centimeters at 24 and 48 hours, similar to that described by Noguera et al.[13] This procedure was repeated in triplicate.

The formulated topical agent with the best results was tested with the wild strains, repeating the same procedure described above. Readings were taken at 24 and 48 hours.

Clinical phase

Participants

The sample consisted of adult patients of legal age, who attended the outpatient clinic of the General Surgery Service of the Central Hospital of Maracay, Aragua state, for postoperative follow-up of abdominal surgery and voluntarily agreed to participate in the study. Work with post-operative patients of abdominal surgery with superficial surgical site infection was done as inclusion criteria, diagnosed in the postoperative follow-up, starting 72 hours after the surgical intervention with ages between 18 and 50 years, without associated chronic diseases and with a body mass index lower than 30 kg/m^2

The patients were characterized from the sociodemographic and clinical point of view, through a survey and direct evaluation of the surgical site. The sociodemographic variables evaluated were: sex, age, country region, social stratum, according to Graffar modified by Méndez-Castellano.[14] The clinical characteristics were wound size in centimeters, presence or absence of signs such as fever, secretions, and phlogosis.

Technique

Prior to the patient trial, the skin irritation test of the topical agent was performed. For this, 10 healthy volunteers with no lesions participated to whom 2g of ointment or cream was applied to the skin on the back of the hand and allowed to act for 2 hours. Then they were asked to wash their hands to remove the topical agent and it was verified whether any undesirable change in color or morphology occurred in the skin of the hands. Once it was verified that there was no irritation, the clinical trial was carried out with the patients, who were randomly distributed into two groups under a double-blind

Citation: Capanna PVDVC, Lugo FOC, Illera MAS, et al. Assessment of a *Mangifera indica* seed extract in topical cream as an adjuvant treatment on surgical site infections. *Int Clin Pathol J.* 2026;11(1):46–52. DOI: 10.15406/icpjl.2026.11.00227

design. The experimental group included patients who were given standardized antibiotic therapy combined with the mango seed extract dressing selected based on the in vitro studies. The control group consisted of patients who received standardized antibiotic therapy along with the control or placebo dressing formulated with ethanol.

The product was applied as wound dressings with supervised applying frequency of every-other-day. Treatment began from the moment the infection was diagnosed and continued until its complete resolution with a final check performed on the tenth day. The wound dressings for all patients, both the experimental and control groups, were performed as described here: the patients were taken to an aseptic space where the surgical wound was uncovered, and with sterile gloves the secretions present in the wound were drained using sterile gauze and applying pressure in a cephalocaudal direction, aiming at favoring the extraction of the exudate. The maneuver was repeated until minimum or no drainage was obtained. Subsequently, surgical cleansing of the wound was performed using 0.2% chlorhexidine as a sweeping solution, wound cleansing with 0.9% sterile solution to remove the chlorhexidine. The solution was applied to a sterile gauze and cleansing was performed by circular friction in a single direction, following a unidirectional pattern to avoid recontamination of previously disinfected areas.

Once the wound was clean, a sterile gauze was impregnated with the selected topical agent (also sterile) using a new, previously sealed applicator. The impregnated gauze was placed over the entire wound. Finally, it was covered with a second dressing; sterile, dry and secured with medical adhesive.[15]

Patients in both groups underwent clinical evaluations three times a week during the study period, concurring with scheduled visits for the daubing of the experimental or placebo dressing. A multidisciplinary team, composed of residents and specialists belonging to the General Surgery service of the Hospital Central de Maracay, carried out an exhaustive assessment of the wounds at each visit. This assessment included the observation of clinical signs such as the quantity and characteristics of the secretion, the presence of erythema, heat, pain and swelling, the presence of hyperthermia and/or chills, as well as the evaluation of the appearance and evolution of granulation tissue and re-epithelialization.

The information per patient was systematically recorded in a database for later analysis. The treatment of each patient ended when the complete resolution of the infection was achieved; defined by the disappearance of clinical signs of infection and evidence of the start of the healing process. Time was also important data recorded per patient.

Statistical analysis

The data obtained at the *in vitro* phase was analyzed by descriptive statistics. The average and standard deviation were calculated and the topical formulation with the best results in inhibiting the growth of microorganisms was selected. For phase two, the data on the socio-epidemiological characteristics of the patients were analyzed through descriptive and inferential statistics. The Fridman test using Chi-square (X^2) as a test statistic was used for categorical or nominal variables, and the student's t-test for numerical variables, to determine the homogeneity of the patient groups. To evaluate the effect of the topical formulation with *M. indica*, inferential statistics were applied with the Fisher's exact test using X^2 as a test statistic in the case of categorical variables and the student's t-test for numerical variables. A significance level of 5% (α=0.05) was used and the statistical program EPI INFO 7.2.

Results

Description of the topical dressings

The appearance of the prepared dressings was homogeneous, brownish in color, with those under a formulation at 10% being a lighter shade and those at 15% being darker. The pH was within the physiological range of the skin, with the lowest being 6.3 and the highest 6.9. The difference between the two types of formulated dressings was basically their water content. The ointments did not contain water in their composition, while the creams had around 50% water according to the description of the manufacturing laboratory.

In vitro antibacterial activity

After 24 hours of incubation, inhibition halos of growth were revealed for both *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, obtaining the highest average inhibition with the 15% cream (Table 1). By 48 hours, no inhibition was observed with any of the topical dressing's formulations.

For the inhibition test with bacteria from clinical or wild isolates it was chosen to use only the cream-type topical dressing, at the established concentrations of 10% and 15% respectively, because they showed better results than the ointments. The bacteria were identified as *Burkholderia cepacia* and *Pseudomonas aeruginosa*, both considered high-risk nosocomial microorganisms for patients. As in the previous case, the inhibition halos were only observed at 24 hours (Table 1).

Table 1 Effect of the formulated topical dressings on the inhibition of bacterial growth evaluated through the diameter of the inhibition halo after 24 hours of incubation

Bacteria	Average and deviation of the inhibition halo expressed in mm			
Certified strains	Ointment 10 %	Ointment 15 %	Cream 10 %	Cream 15 %
E. coli ATCC 25922	8.0 ± 0.1	13.0 ± 0.7	11.5 ± 0.1	15.0 ± 1.4
S. aureus ATCC 25923	7.0 ± 1.4	11.0 ± 1.2	9.5 ± 0.5	14.0 ± 1.0
Wild strains				
B. cepacia	----	----	16.5 ± 2.1	15.7 ± 0.7
P.aeruginosa	----	----	14.5 ± 0.7	15.0 ± 1.0

Note: On wild strains only the creams were tested, which is why there is no information on the effect of ointments.

At this stage it was concluded that the 15% cream showed the best results for the inhibition of the tested strains.

Clinical trial

A total of 20 patients who met the inclusion criteria were included; with an average age of 31.7±9.9 years; 50% female and 50% male,

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the majority from Aragua state (90%) and social stratum III (60%) according to the modified Graffar scale.

Two study groups, experimental and control, were formed with 10 patients each and with statistically similar sociodemographic and clinical characteristics ($p>0.05$), thus confirming that they were homogeneous and comparable for the evaluation of the topical dressings (Table 2 and 3).

Table 2 Sociodemographic characteristics of the study groups, composed of patients with surgical site infection assessed by the General Surgery service of the Hospital Central de Maracay, Aragua State, Venezuela, 2024

Variables	Experimental group		Control Group		
Cuantitative Variables	**Avg ± SD**		**Avg ± SD**		**p**
Age (years)	32.8 ± 12.6 yrs		30.6 ± 7.1 yrs		0.639
Cualitative Variables	n	%	N	%	p
Sex					1
Female	5	50	5	50	
Male	5	50	5	50	
Origin					1
Aragua	9	90	10	100	
Carabobo	1	10	0	0	
Social strattum acc. to Graffar					0.891
I	0	0	0	0	
II	1	10	1	10	
III	6	60	4	40	
IV	2	20	4	40	
V	1	10	1	10	

Statistical tests: Student's t-test for quantitative variables and X^2 for qualitative variables with an α value of 0.05.

Table 3 Clinical characteristics of the study groups, composed of patients with surgical site infection assessed by the General Surgery service of the Hospital Central de Maracay, Aragua State, Venezuela in the period from January to June 2024

Variables	Experimental group		Control Group		
Cuantitative Variables	**Avg ± SD**		**Avg ± SD**		**p**
Tamaño de la herida (cm)	8.2 ± 2.1 cm		7.6 ± 1.2 cm		0.443
Cualitative Variables	**n**	**%**	**N**	**%**	**p**
Hypertermia					1
Present	3	30	3	30	
Absent	7	70	7	70	
Phlogosis					1
Present	10	100	10	100	
Absent	0	0	0	0	
Secretion					0.474
Present	10	100	8	80	
Absent	0	0	2	20	
Wound contamination					0.35
Visible	2	20	5	50	
Not visible	8	80	5	50	

Statistical tests: Student's t-test for quantitative variables and X^2 for qualitative variables with an α value of 0.05

The follow-up of patients after the application of the topical dressing treatment carried out at 2, 6, and 10 days, allowed to evidence the effect of the extract. Patients who were treated with the cream with 15% mango extract exhibited substantial improvement in their clinical condition on the sixth day. 80% of the patients no longer presented phlogosis, 100% did not have secretion and in 80% it was observed that the process of skin granulation had begun. By the tenth day 100% of the patients no longer presented either phlogosis or secretion; and granulation and re-epithelialization of the surgical site had been achieved. In contrast to the control group, signs of granulation or re-epithelialization associated with skin recovery were not observed on the sixth day, but until the tenth day (Figure 1).

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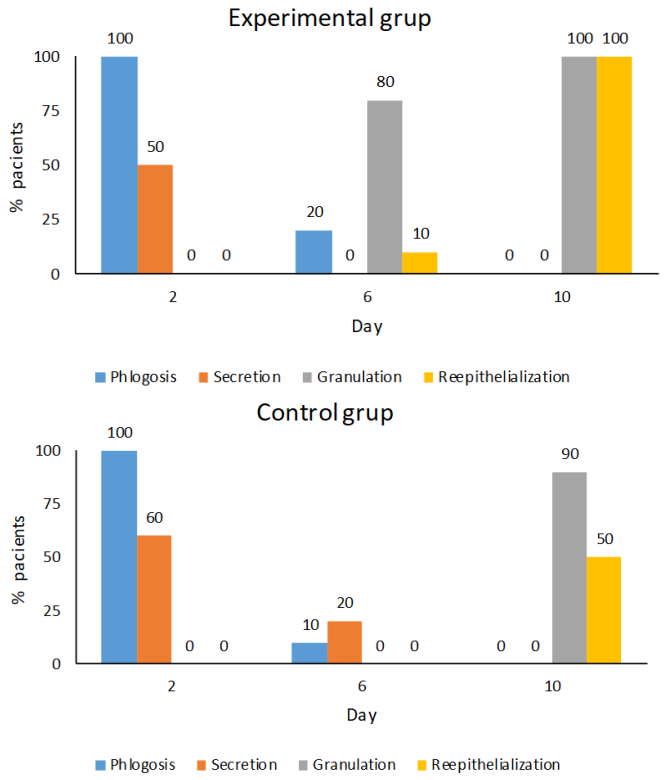

Figure 1 Evolution of post-operative patients of abdominal surgery with superficial surgical site infection, based on the presence of phlogosis, secretion, granulation, and re-epithelialization, during 2, 6, and 10 days after treatment.

Photographic evidence of the evolution of a patient from the experimental group demonstrated complete wound healing on the tenth day of treatment (Figure 2).

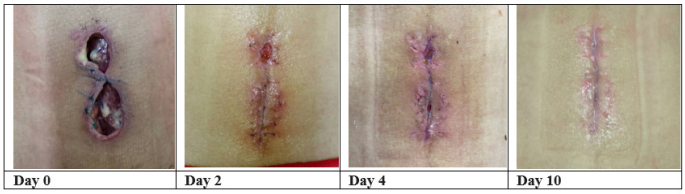

Figure 2 Evolution of an abdominal surgery post-op patient with surgical site infection treated with 15% mango extract cream.

When comparing the groups with Fisher's exact test, a significant difference was obtained among the groups based on re-epithelialization on the tenth day (p=0.032). This matched the fact that the time to resolution of the infection also resulted significantly different; in the experimental group, the average time was 6.0±0.9 days, while for the control group it was 7.6±1.1 days (p=0.0035).

Discussion

In a study by Zivković et al.,[16] it is pointed out that one of the challenges of using medicinal plants is the low bioavailability of bioactive compounds. In the specific case of mango and mangiferin, low solubility and absorption condition its use. However, the formulated cream turned out to be an excellent medium that favored the release and absorption of the bioactive compounds present in the mango extract. In general, creams are, of all semi-solid formulas, those with the highest water content (more than 50%) and are a mixture of water and fats (non-miscible) that mix thanks to the action of emulsifiers that produce a stable mixture.[17]

The *in vitro* evaluation of the antimicrobial activity of the extract using certified bacterial strains (*S. aureus* and *E. coli*) showed the presence of inhibition halos for both the vehicles and the pure extract, which indicates the presence of antibacterial activity in both presentations. These findings are consistent with those reported by Noguera et al.,[11] who demonstrated the antimicrobial activity of *M. indica* extracts of species from Venezuela and, in turn, with the data obtained by Amgad et al.,[9] where it was evidenced that, despite the incorporation of the extract into a topical vehicle, these continued to provide antimicrobial activity.

The antimicrobial effect shown by the cream that had the extract incorporated can be attributed to its high content of bioactive phytochemical compounds, mainly polyphenols (gallic acid, ellagic acid, pyrogallol and mangiferin), condensed and hydrolyzable tannins, and flavonoids such as quercetin and isoquercitrin.[18,19] These compounds act through multiple synergistic mechanisms, as for example in the case of phenolic compounds and flavonoids, due to their amphiphilic and lipophilic nature, they insert themselves into the lipid bilayer of the bacterial cell membrane altering permeability and causing its membrane depolarization.[20] In the case of tannins, they have a high affinity for extracellular and intracellular proteins, which produces an interference with the activity of transpeptidases necessary for peptidoglycan polymerization. Additionally, some tannins such as those derived from hydroxybenzoic acids (gallic and ellagic acid) act as potent metal chelators.[21] Mangiferin and gallic acid suppress the expression of genes dependent on acyl-homoserine lactones (AHL) in Gram-negatives and autoinducing peptides (AIP) in Gram-positives, preventing the structuring of biofilms in strains such as *Staphylococcus aureus*,[22] *Serratia marcescens*,[23] and *Pseudomonas aeruginosa*.[24]

The findings in this investigation however, demonstrate greater diameters in the inhibition halos compared to those from the reference strains. Regarding the pharmaceutical presentation, the results of this study differ from those reported by Amgad; since we observed greater efficacy of the cream presentation compared to that of the ointment. Based on the data extracted from this phase of the investigation, the optimal vehicle and concentration were chosen for future phases of the project. As an additional determination of antimicrobial activity, a second *in vitro* assay of the selected topical dressing (10% and 15% cream) was performed on nosocomial and wild strains, where the presence of notably large inhibition halos was demonstrated, ratifying the activity of the cream also against these germs causing infections in the hospital environment.

In accordance with the results obtained in the *in vitro* phase, the safety and the ability of the topical dressing to inhibit the development of bacteria of different nature and origin were verified. Therefore, a protocol was established for the in vivo application on patients of the selected topical dressing (15% cream). With the goal of stating a causal correlation between the application of the topical dressing and the resolution of the infection, a randomized experimental design was used. This design allowed ensuring that the differences observed between the groups were due to the effect of the product and not to other variables, and consequently, increase the internal validity of the study.

The sample was composed of a total of 20 adult patients. The evaluation of demographic variables revealed an equitable distribution by gender, which indicates that no significant association was observed between sex and the development of SSI. Regarding geographical origin, 95% of the participants resided in Aragua state. This result is consistent with the location of the research center, which is located

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in this same region. The results showed a significant presence of secretion in 80% of the cases and local inflammatory signs in 100% of the participants. These findings are consistent with those reported by Zabalgo,[25] who points out that secretion and local phlogosis are characteristic clinical manifestations of surgical site infections due to its pathophysiology. Zabalgo highlights those systemic signs, such as fever, are usually absent or less pronounced in this type of infection, which is confirmed in our study, where only 30% of the participants presented this sign on day 0 of evaluation. While in subsequent evaluations, performed on days 2, 6, and 10, where the presence of hyperthermia and/or chills was evaluated, a complete absence of these symptoms was observed in 100% of the population.

The analysis of the surgical wounds revealed an average size of between 7 and 8 centimeters in length, consistent with the type of interventions performed and the average size of laparotomy wounds. Regarding the state of the wound at the time of evaluation, it was observed that 65% were classified as clean, while 35% presented signs of contamination. These results indicate that, although wound cleanliness is a crucial factor in the prevention of infections, the presence of contamination signs is not a necessary condition for the development of a surgical infection. Through rigorous clinical observation and exhaustive follow-up of the participants, the results demonstrated a significantly faster resolution of the infection in the experimental group compared to the control group. In the experimental group, an almost complete decrease (80%) of the signs of phlogosis was observed by the sixth day of follow-up, while in the control group none of the patients presented this improvement, which indicates that the resolution of the infection began early in the experimental group as the signs of local infection began to disappear. Likewise, the secretion disappeared completely in the experimental group on the sixth day, in contrast to the control group, demonstrating that the experimental group had an early onset of infection control and the beginning of tissue repair.

The healing kinetics were also notably enhanced in the experimental group, with 80% of the patients showing granulation tissue on the sixth day, while in the control group no patient presented this improvement. Re-epithelialization was completed in 100% of the patients in the experimental group on the tenth day, compared to only 50% of the control group. These findings demonstrate the complete resolution of the infection and the start of the healing process of the previously affected tissue. These findings indicate that the experimental treatment significantly speeded the resolution of the infection and the healing process; surpassing the control group in all evaluated parameters. This information is supported by evaluating the analysis of the time to resolution of the infection, which showed a statistically significant difference between both groups. The experimental group showed an average resolution time of 6 days, while the control group required 7.6 days. It is important to note that due to limitations of the hospital center where the study was carried out, the socioeconomic conditions of the patients, and limitations of our study, it was not possible to perform bacteriological culture on every evaluated pacient.

These results confirm the preeminence of the experimental topical dressing in quickening the resolution of the infection and the healing process, thus obtaining promising and satisfactory results that confirm the hypothesis raised in this study and generate new questions for future research. Therefore, the use of the studied topical dressing is recommended for the elaboration of a new protocol in the presence of superficial surgical site infections, as well as to start a new line of research focused on the efficacy of MI extract as a healing agent, this aims at obtaining more competent products to ease the local management of surgical wound infections, a fact that will directly favor the recovery of the patient's health.

Conclusions

The topical cream formulated with 15% mango seed (*M. indica*) extract; proved to be an efficient adjuvant for the treatment of patients with SSI; since it improved the times of granulation, re-epithelialization, and general healing of the patients. Consequently, it can be affirmed that the extraction process used and the formulated cream favored the bioavailability of compounds present in the mango seed, so that all its medicinal potential could be seized.

Acknowledgments

The authors extend their deepest thanks to the general and laparoscopic surgery service of the Central Hospital of Maracay and its specialists and residents.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Funding

None.

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Citation: Capanna PVDVC, Lugo FOC, Illera MAS, et al. Assessment of a *Mangifera indica* seed extract in topical cream as an adjuvant treatment on surgical site infections. *Int Clin Pathol J.* 2026;11(1):46–52. DOI: 10.15406/icpjl.2026.11.00227