







Research Article

# Antimicrobial and Wound Healing Potentials of Methanol Extract of *Salvia Officinalis* (Common Sage) Leaf

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## Abstract

Background: Food-borne microbial infections continue to pose a significant public health burden, particularly in low-income nations, where they contribute substantially to morbidity and mortality. Their management is increasingly hampered by the spread of multidrug-resistant pathogens, hence the aim for the study. Methods: The study evaluated the antimicrobial and wound healing potentials of methanol extract of *Salvia officinalis* leaf (MESoL). Antimicrobial activity was assessed using the Mueller-Hinton agar well diffusion method against selected bacterial strains. Wound healing efficacy was investigated using an excision wound model in Wistar rats. Data were analyzed using one-way ANOVA with Dunnett's post hoc test. Results: MESoL and 1% silver sulfadiazine demonstrated sensitivity against *Staphylococcus aureus*, *Corynebacterium ulcerans*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*. The minimum bactericidal concentration (MBC) values of MESoL for these organisms were 30mg/ml, 30mg/ml, 60mg/ml, 15mg/ml and 60mg/ml respectively. In vivo studies revealed a significant reduction in wound area ( $p < 0.05$ ,  $p < 0.001$ ) over 11 days compared to control groups. The extract promoted wound contraction and tissue repair, indicating both antimicrobial and healing potential. Conclusion: The methanol extract of *Salvia officinalis* leaf exhibits dual functions as a natural antimicrobial and wound-healing agent. These findings support its potential application as a complementary therapy for microbial infections and wound management.

## Keywords

Antimicrobial, Cream Formulation, *Salvia Officinalis* Leaf, Topical, Wound Healing

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## 1. Introduction

Millions of people worldwide suffer from pathogenic infections caused by organisms such as *Staphylococcus aureus* and *Salmonella typhi*, which are frequently transmitted through contaminated food and water. These foodborne pathogens represent major contributors to elevated morbidity and mortality rates in developing countries. Herbal medicines are frequently employed to manage gastroenteritis and other bacterial infections. Therefore, investigating plant-derived antimicrobials may provide novel strategies to combat antibiotic resistance [1-3]. Medicinal plants can inhibit bacterial growth through mechanisms distinct from those of conventional antibiotics, thereby assisting in the management of resistant infections. Several studies have documented the screening of plants for antimicrobial properties [3-6], often revealing structurally complex compounds that can be synthesized by chemists to enhance antimicrobial activity.

*Salvia officinalis* (Common sage), a member of the *Lamiaceae* family is an evergreen aromatic perennial shrub utilized both as a culinary herb and in traditional medicine [7-11]. According to researchers, *Salvia officinalis* may be employed to treat various human diseases, even at elevated doses, particularly because it lacks thujone, a compound associated with toxicity at high concentrations [12]. The essential oil of *Salvia officinalis* exhibits multiple biological activities, including anti-proliferative, antimicrobial, anti-inflammatory, antioxidant, antiviral, and insecticidal effects [13-16]. The leaves contain bioactive constituents such as tannins, saponins, and flavonoids, which possess antioxidant properties and are considered relatively safe. These compounds may serve as natural agents to protect the liver during parasitemia-induced hepatotoxic injury [11, 17]. Currently, there is limited scientific data regarding the antimicrobial and wound-healing properties of this plant in developing countries such as Nigeria. Therefore, this study aimed to investigate these potentials using the methanol extract of *Salvia officinalis* leaf.

## 2. Materials and Methods

### 2.1. Drugs and Chemicals

All chemical-Delete All chemicals utilized in this study were of analytical grade. These materials included 1% silver sulphadiazine (Salutes Pharmacy GmBh, Germany). Silymarin tablets 140mg film coated (Micro Labs Ltd, India) and Lidocaine injection 2% (Erica Life Science Ltd, UK).

### 2.2. Collection, Identification and Preparation of Plant Extract

The *Salvia officinalis* (Sage) plants were collected from Vom-Jos, Plateau State-Nigeria and identified/authenticated by a botanist (Michael, Ozioma Emmanuel) at Delta State

University, Abraka. The plant was successfully propagated at our garden facility at Asaba (Figure 1). A voucher specimen (DELSU#134) was deposited for future reference. The collected leaves were cut into small pieces, dried at a temperature of 30 - 40°C for 21 days, then pulverized to a fine powder using standard procedures. A 50-gram sample of the powdered material was mixed with 400 mL of 95% methanol in a conical flask and shaken intermittently for three days at room temperature. The mixture was filtered through muslin cloth and subsequently concentrated using a vacuum evaporator. The resulting extract was stored in an airtight container under refrigerated conditions.



**Figure 1.** Propagated *Salvia officinalis* (Common Sage) leaf plant at Asaba garden.

### 2.3. Animal Experiment

The study received approval from the Research and Ethics Committee, Faculty of Science, Delta State University, Nigeria, (REC/DELSU/FOS/2021/02). Healthy male Wistar rats weighing between 150-250 grams were obtained from the University's Animal House facility. The animals were housed under appropriate conditions, fed standard growers' mash and water *ad libitum*. All animal handling procedures adhered to International Council for Laboratory Animal Science (ICLAS) and Delta State University protocol. The methanol extract was suspended in distilled water at a concentration of 5000 mg/kg for oral administration.

### 2.4. Acute Toxicity Tests (LD<sub>50</sub>)

An acute toxicity study (LD<sub>50</sub>) of the methanol extract of the *Salvia officinalis* leaf was conducted in two phases as recently described by Lorke [18] and reported recently by Evinemi et al., [19, 20]. Nine (9) mice were used in the first phase that comprised of three groups (n = 3). They were given orally, with the methanol extract of the Sage leaf at doses of 10, 100 and 1000mg/kg body weight. Then, the animals were observed for 24 h for signs of toxicity and mortality. The second stage (phase) of three (3) mice (n=1) received specific doses of 1600, 2900 and 5000 mg/kg following the results of the first stage. The lethal dose (LD<sub>50</sub>) was calculated using the

formula below:

$$LD_{50} = \sqrt{\text{minimum lethal dose} \times \text{maximum tolerated dose.}}$$

## 2.5. Cream Extract Formulation

The formulation components included sorbitan monostearate and stearic acid which were melted in paraffin and cooled at 54°C. The initial quantity of the extract used was 30mg. Ten milliliter (10ml) volumes were made as needed using the formula RV/O, (where R = Required concentration, V = Required volume, O = Original concentration). Methanol extract concentrations of 1%, 5% and 10% (w/w) were made and mixed with propylene glycol 4.02% (w/w), glycerin 4.13% (w/w) and 10ml of sterile distilled water. The mixtures were heated until completely dissolved and then allowed. The same procedure was repeated for 5% and 10% concentrations. Additional components including acetyl alcohol 3.56% (w/w), stearic acid 4.50 (w/w), olive oil 5.78% (w/w), span 60 1.78% (w/w) and tween 80 (0.75% w/w) were also mixed, heated in a beaker for dissolution, stirred together and allowed to cool. The Creams were then separated in different universal container for use, for the wood healing studies.

## 2.6. Antimicrobial Assessment

### 2.6.1. Microorganisms and Growth

Six test microorganisms were utilized in this study: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium ulcerans*, *Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typhi*. These clinical isolates were obtained from National Veterinary Research Institute, Vom-Jos. Plateau State. The standard antibiotic control employed was 1% silver sulfadiazine. Methanol extract of *Salvia officinalis* leaves (0.6g) was dissolved in 10ml of sterile distilled water to achieve a concentration of 60mg/ml. This served as the initial concentration for the study. The medium used for the growth medium was Mueller Hinton agar. It was prepared according to the manufacturer's instruction and sterilized at 121°C for 15min. This was poured into sterilized Petri-dish, allowed to cool and solidify completely. The agar well diffusion method was employed for antimicrobial testing. A standard inoculum (0.1ml) was evenly spread across each agar plate surface. A 6-mm diameter well was aseptically cut at the center of each plate, and 0.1ml of extract solution (60mg) was carefully added to each well. The inoculated plates were subsequently inoculated at 37°C for 24hours. Following inoculations, zones of inhibition surrounding each well were measured in millimeters using appropriate measuring instruments.

### 2.6.2. Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration was determined using the broth dilution method as described by Celiktas et al. [21] and adapted by Sheidu et al. [4]. Mueller-Hinton broth

(10 mL) was sterilized at 121°C for 15 minutes and subsequently cooled to room temperature. McFarland turbidity standards (0-5) were prepared according to established protocols. Normal saline (10 mL) was inoculated with the test organism and incubated at 37°C for 6 hours. The suspension was then serially diluted until the turbidity matched the McFarland standard, achieving approximately  $1.5 \times 10^8$  CFU/mL. Two-fold serial dilutions of MESoL extract were prepared in sterile broth to yield final concentrations of 60, 30, 15, 7.5, and 3.75 mg/mL. Subsequently, 0.1 mL of the standardized test organism suspension was added to each concentration. The inoculated tubes were incubated at 37°C for 24 hours and examined for turbidity. The lowest concentration exhibiting no visible turbidity was recorded as the minimum inhibitory concentration.

### 2.6.3. Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined following the methodology described by Celiktas et al. [21] and Sheidu et al. [4]. Contents from the MIC determination tubes were subcultured onto Mueller-Hinton agar plates and incubated at 37°C for 24 hours. Following incubation, plates were examined for colony growth. The lowest extract concentration demonstrating complete absence of colony growth was designated as the minimum bactericidal concentration.

## 2.7. Wound Healing Studies

The excision wound model described by Nayak et al. [22] and adapted by Sheidu et al. [4] was employed for this investigation. Wistar rats were weight-matched and randomly assigned to six experimental groups (n=5 per group). Following surgical excision, animals were housed individually to prevent cannibalism. The prepared creams were applied topically to the dorsal excision sites. Prior to treatment administration, the dorsal fur was carefully shaved, and excision margins were delineated using dilute picric acid solution. Rats were anesthetized with lidocaine, and full-thickness excision wounds measuring 1 cm in width were created using sterile forceps, surgical blades, and scissors under aseptic conditions.

The experimental groups were organized as follows:

Group I (Control): Base cream application only

Groups II, III, and IV: 1%, 5%, and 10% MESoL cream formulations, respectively

Group V (Synergism): Combined 1% silver sulfadiazine and 10% extract

Group VI (Standard): 1% silver sulfadiazine alone

The treatment protocol extended over 11 days. Wound area measurements were obtained on days 3, 5, 7, 9, and 11 using precision calipers, thread, and rulers. Animals were monitored for an additional two weeks post-treatment to assess potential toxicity indicators. The wound healing percentage was calculated using the following formula:

$$\text{Wound Healing (\%)} = \frac{\text{Initial wound area} - \text{Unhealed}}{\text{Initial wound area}} \times 100$$

wound area) / Initial wound area] × 100

## 2.8. Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test, executed through SPSS software. Statistical significance was established at  $p < 0.05$ .

## 3. Results

### 3.1. Effects of Methanol Extract of *Salvia officinalis* Leaves (MESoL) on the Studied Microorganisms

Both MESoL and 1% silver sulfadiazine demonstrated sensitivity against *Staphylococcus aureus*, *Corynebacterium ulcerans*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*. However, *Streptococcus pyogenes* exhibited resistance to both antimicrobial agents (Table 1).

### 3.2. Effects of MESoL and 1% Silver Sulfadiazine on Zone of Inhibition on Test Microorganism

The zone diameter measurements (mm) for MESoL were as follows: *S. aureus* (20 mm), *S. pyogenes* (0 mm), *C. ulcerans* (22 mm), *E. coli* (18 mm), *K. pneumoniae* (28 mm), and *S.*

*typhi* (20 mm). Corresponding values for 1% silver sulfadiazine were 0, 0, 30, 30, 32, and 28 mm, respectively (Table 2).

The results of the zone of inhibition of the MESoL against test microorganisms were: *S. aureus* -20, *S. pyogenase* -0, *C. ulcerans* -22, *E. coli* -18, *K. pneumonia* -28 and *S. typhi* -20 in millimeters. Also the results for 1% silver sulfadiazine against the same microorganisms were 0, 0, 30, 30, 32 and 28 millimeters respectively in the stated order of the microorganisms above (Table 2).

### 3.3. Effects of Minimum Inhibitory Concentration (MIC) and Antimicrobial Susceptibility Using Mesol

MIC analysis revealed that *S. aureus*, *C. ulcerans*, *E. coli*, and *S. typhi* were effectively inhibited at 15 mg/mL, with turbidity and slight growth observed at 7.5 mg/mL and moderate turbidity at 3.75 mg/mL. *S. pyogenes* demonstrated no inhibition across the tested concentrations. *K. pneumoniae* exhibited an MIC of 7.5 mg/mL, with visible turbidity at 3.75 mg/mL (Table 3).

### 3.4. Effects of Minimum Bacterial Concentration (MBC) on Test Microorganisms Using Mesol

The MBC values for MESoL were determined as follows: *S. aureus* (30 mg/mL), *C. ulcerans* (30 mg/mL), *E. coli* (60 mg/mL), *K. pneumoniae* (15 mg/mL), and *S. typhi* (60 mg/mL). No bactericidal effect was observed against *S. pyogenes* (Table 4).

**Table 1.** Antimicrobial susceptibility effect of methanol extract of *Salvia officinalis* leaf (MESoL) and 1% silver sulfadiazine.

Test Organism	MESoL	1% Silver Sulfadiazine
<i>Staphylococcus aureus</i>	Sensitive	Sensitive
<i>Streptococcus pyogenase</i>	Resistant	Resistance
<i>Corynebacterium ulcerans</i>	Sensitive	Sensitive
<i>Escherichia coli</i>	Sensitive	Sensitive
<i>Klebsiella pneumonia</i>	Sensitive	Sensitive
<i>Salmonella typhi</i>	Sensitive	Sensitive

**Table 2.** Effects of methanol extract of *Salvia officinalis* leaf (MESoL) and 1% silver sulfadiazine on zone of inhibition on test organism Zone of Inhibition (mm) Test Organism MESoL 1% Silver Sulfadiazine.

Test Organism	MESoL	1% Silver Sulfadiazine
<i>Staphylococcus aureus</i>	20	0
<i>Streptococcus pyogenase</i>	0	0
<i>Corynebacterium ulcerans</i>	22	30

Staphylococcus aureus	20	0
Escherichia coli	18	30
Klebsiella pneumonia	28	32
Salmonella typhi	20	28

**Table 3.** Minimum inhibitory concentration (MIC)(mg/ml) and antimicrobial susceptibility effect of MESoL against test organism.

Test Organism	60	30	15	7.5	3.75
Staphylococcus aureus	-	-	0*	+	++
Streptococcus pyogenase	-	-	-	-	-
Corynebacterium ulcerans	-	-	0*	+	++
Escherichia coli	-	-	0*	+	++
Klebsiella pneumonia	-	-	-	0*	+
Salmonella typhi	-	-	0*	+	++

- = No turbidity (No growth), 0\*= Minimum inhibition concentration,  
+ = Turbidity (slight growth), ++ = Moderate turbidity.

**Table 4.** Minimum bacterial concentration (MBC) of extract of *Salvia officinalis* leaf on test organism.

Test Organism	60	30	15	7.5	3.75
Staphylococcus aureus	-	0*	+	++	+++
Streptococcus pyogenase	-	-	-	-	-
Corynebacterium ulcerans	-	0*	+	++	+++
Escherichia coli	0*	+	++	+++	++++
Klebsiella pneumonia	-	-	0*	+	++
Salmonella typhi	0*	+	++	+++	++++

- = No turbidity (No growth), 0\*= Minimum inhibition concentration,  
+ = Turbidity (slight growth of colonies), ++ = Moderate growth of colonies, +++ = Heavy growth of colonies, ++++ = Dense growth of colonies

### 3.5. Effects of Topical Application of MESoL Cream on Excision Wound in Wister Rats Treated for 11days

On day 3, the 1% MESoL cream demonstrated no significant difference ( $p > 0.05$ ) compared to the base cream ( $0.82 \pm 0.03$  vs.  $0.89 \pm 0.02$ ). In contrast, the 5% and 10% cream formulations significantly reduced wound area ( $0.60 \pm 0.06$  and

$0.46 \pm 0.04$ , respectively;  $p < 0.001$  vs. base). The standard drug (1% silver sulfadiazine) exhibited complete healing potential at day 3. On day 5, the 1% extract achieved approximately 95% healing ( $p < 0.05$ ), while both 5% and 10% concentrations achieved complete healing ( $p < 0.001$ ). On days 7 and 9, all extract concentrations demonstrated complete healing ( $p < 0.001$ ). By day 11, all treatment groups showed significant wound closure ( $p < 0.05$ ) (Table 5).

**Table 5.** Effects of topical application of *Salvia officinalis* cream extract on excision wound in Wistar rats treated for 11 days.

Cream (%w/w)	Reduction in diameter of Wound				
	D3	D5	D7	D9	D11
Base	0.89 ± 0.02	0.07 ± 0.04	0.66 ± 0.04	0.52 ± 0.02	0.43 ± 0.03
Ext.1%	0.82 ± 0.03	0.60 ± 0.04	0.48 ± 0.04	0.18 ± 0.03	0.08 ± 0.02
Ext.5%	0.60 ± 0.06	0.42 ± 0.04	0.22 ± 0.04	0.16 ± 0.03	0.08 ± 0.02
Ext.10%	0.46 ± 0.04	0.30 ± 0.06	0.18 ± 0.02	0.12 ± 0.03	0.10 ± 0.02
1% SS	0.06 ± 0.06	0.40 ± 0.04	0.22 ± 0.02	0.14 ± 0.01	0.08 ± 0.02

Values expressed as Mean ± SEM, n = 5, ns = Not significant, a = p < 0.05, b = p < 0.001. Analysis by one way ANOVA followed by, Dunnet's t-test. SS = Silver Sulfadiazine

## 4. Discussion

The acute toxicity findings demonstrate that the methanol extract of *Salvia officinalis* leaves exhibits an acceptable safety profile, as no adverse effects were observed in Wistar rats. This supports the extract's potential therapeutic application without significant toxicological concerns. Furthermore, the topical cream formulation displayed wound healing activity comparable to the reference drug (1% silver sulfadiazine), confirming its efficacy in tissue repair. These results are consistent with earlier reports by John-Africa et al. [23] and Sabale et al. [24], who demonstrated similar wound-healing properties in other medicinal plants.

The antimicrobial evaluation revealed substantial activity against *S. aureus*, *C. ulcerans*, *E. coli*, *K. pneumoniae*, and *S. typhi*, as demonstrated by inhibition zones, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values. These findings align with those of Josephus et al. [3], who reported comparable efficacy against the same pathogens using a different plant species. Our results also correspond with studies conducted by Tkachenko et al. [25], Anyekeke et al. [1], and Belattar et al. [26], who investigated antimicrobial effects in various herbal extracts.

The observed antimicrobial activity of the methanol extract of *S. officinalis* (MESoL) also corroborates the findings of [12], who reported that essential oil and crude organic extracts of *S. officinalis* leaf inhibited the growth of *S. aureus*, *K. pneumoniae*, and *E. coli* in Nepal. This is further supported by [27], who highlighted the phytochemical, antioxidant, and antimicrobial activities of *S. officinalis* leaf in Saudi Arabia. Likewise, our results agree with [16], who demonstrated the bactericidal activity of *S. officinalis* essential oil cultivated in Morocco, and with [28], who confirmed the antimicrobial efficacy of ethanol extracts of *S. officinalis* against *Proteus mirabilis*.

The MBC results confirmed that *S. aureus* and *K. pneumoniae* isolates exhibited the highest susceptibility, while *E. coli*

and *S. typhi* showed the greatest antimicrobial activity. These observations are consistent with the reports of [1, 4, 29-31], despite their studies focusing on different medicinal plants. The antimicrobial and wound-healing potentials observed may be attributed to the phytochemical constituents of *Salvia officinalis*. Vidhya et al. [32], highlighted that plants synthesize aromatic substances, such as phenolic compounds and oxygen-substituted derivatives, contribute to antimicrobial and antioxidant activity. Similarly, Rodendo et al. [33], emphasized that these compounds serve as defense mechanisms against microbial invasion, while terpenoids, quinones, and tannins contribute additional biological functions. Ahmad and Beg [34], further explained that the mode of action of plant-derived agents targets biochemical features unique to pathogens, thereby conferring selective antimicrobial activity. Thus, the presence of bioactive phytochemicals in *S. officinalis* may account for its demonstrated antimicrobial, antioxidant, immunomodulatory, anti-inflammatory, and wound-healing effects, as previously reported by some scholars [6, 27, 35, 36]. Collectively, these properties support its potential role as a therapeutic agent for infection control and wound healing.

## 5. Conclusion

The methanol extract of *Salvia officinalis* leaf demonstrates significant antimicrobial activity and wound-healing potential, presenting a promising therapeutic option for treating microbial infections and enhancing wound repair processes.

## Abbreviations

MESol	Methanol Extract of Salvia Officinalis Leaf
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bacterial Concentration
SS	Silver Sulfadiazine

## Author Contributions

**Azukaego Thomas Hughs Mokogwu:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing

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**Nathaniel Bini:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Writing – review & editing

**Enwa Felix Oghenemaro:** Data curation, Formal Analysis, Funding acquisition, Resources, Software, Supervision, Visualization, Writing – review & editing

## Data Availability Statement

All relevant data are within the manuscript and its supporting information files. Additional data will be available on request according to the journal policy.

## Conflicts of Interest

The authors declare no conflict of interest.

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