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Article

# Potential action of SAGE extracts to prevent the growth of bacteria isolated from patients suffering from diarrhea and one type of cancer cell

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**ABSTRACT**

Now, the trend has begun to use some types of herbs, including salvia sage, in the development of medicines and medicinal drugs instead of synthetic drugs because they are antimicrobial and are considered preservatives against food spoilage. Sage is also an aromatic material used as a food flavoring. This work represented examining the antibacterial impact of the sage extract on four types of pathogenic and drug-resistant bacteria in vitro, like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas eruginosa*, besides *Klebsiella pneumonia*, *Providencia, and Burkholderia*. On the other hand, cytotoxic effects of the ethanol extract of Salvia showed antitumor activity on MCF-7 cells in a dose-dependent manner as the cell viability against MCF-7 cells was decreased with an IC50 of µg/ml. The experimental study of the antibacterial influence of extracted sage plants from ethanol on the evolution of multi-drug resistant bacteria was fulfilled with good diffusion at different concentrations: 50, 150, 200 mg/mL, and microdilution methods. Disclose the impact of the sage methanol extract on MCF-7 cell viability.

In our study, Ethanol extracts of sage in the good diffusion method displayed obvious notable inhibitory against bacterial growth. These results refer to the inhibitory impacts of ethanol extract of sage with MIC (Minimum Inhibitory Concentration)=8 mg/mL owing to *S.aureus*, MIC=5 mg/mL for *E. coli*, MIC=4.4 mg/mL owing to *P. aeruginou*s, MIC=4.2 mg/mL owing to *Burkholderia*, MIC=5.7 mg/mL owing to *Klebsiella pneumonia.* The impact found with *Providencia.* Results indicated a dose-dependently growth inhibition (63.18% at 250 µg $mLG^{1}$). Concerning the antibacterial impact of ethanol extracts of Sage on the multi-drug impervious to bacteria, the use of herbs as a replacement to antibiotics after pharmacological studies for treatment is recommended. Methanol extract of sage exhibited profound cytotoxicity activity against the MCF-7 cell line.

**Keywords:** Salvia sclarea, sage, Antibacterial activity, cytotoxicity, MCF-7 cell line.

## INTRODUCTION

Sage is a perennial green tree of the mint family (Lamiaceae). Its flowers are wonderful and in various colors, of which 17 species were planted in Iran [1] and settled in the Mediterranean region. They were recently used as ornamental plants as well as in alternative medicine. Sage of the salvia officinal type is one of the most important to use in treatments [2]. Many terpenoids in sage teas contribute to their antioxidant, antimutagenic and antidiabetic effects. Salvia is called the sage plant and is used as a culinary herb. It is considered a medicinal plant. It was previously known as Rosemarinus Officinalis. Its flowers are attractive, and its leaves are aromatic, suitable for seasoning foods [3]. Sage contains many oils with antispasmodic, carminative, antiseptic, anticancer, antimicrobial, antioxidant, and free radical scavenging functions [4]. Studies have shown the effectiveness of sage extract on microorganisms in the mouth of a group of school-age children (adolescents) [5]. Natural compounds such as flavonoids and polyphenols found in sage are caffeic acid, carnosic acid, and rosmanic acid, which have anti-inflammatory and antioxidant properties that are why they are used in cosmetics [6]. Silver nanoparticles were synthesized from the sage extract of Salvia type and showed cytotoxic properties on MCF-7 cancer cell lines. Therefore, it can be considered a determinant factor for antitumor agents used in the treatment of MCF-7 breast cancer, which is widely spread in many women worldwide and may extend to other organs such as the liver, bones, brain, and lungs [7,8]. The most significant issue here is producing resistant strains of bacteria. Despite the efforts, large-scale antimicrobial substances have been developed. Medicinal plants can now be used in proportion to the advances in science and technology. Using sage extracting on the bacterial face under vitro conditions is beneficial due to the tolerance of bacteria to common antimicrobial medication and the less harmful side- effects of natural remedies [9].

## MATERIAL AND METHODS

The experimentation has been carried out at Microbiology Research Laboratory, Microbiology Department, 2019.

### Tested Strains:

A clinical investigation on 60 MDR (multi-drug-resistant) S samples, including 10 samples, was conducted. Aureus, 10 samples for MDR P. aeruginosa, 10 MDR Klebsiella, 10 MDR Staph. aureus, 10 MDR Samples Providencia, 10 MDR Samples Burkholderia isolated, including urinary tract infections, breathing disease, ear infections, wounds, abscesses, skin infections, spinal diseases, 10 MDR Burkholderia isolated and sputum infections. The isolation was obtained in Baghdad hospitals. Biochemical experiments were carried out after samples were obtained to diagnose the bacterial genera and species.

### Antibiotics susceptibility test:

The test employed an agar disc propagation or Kirby-Bauer process, as suggested by NCCLS on bacterial strains. This examination contains antibiotic disks such as erythromycin, ampicillin, amoxicillin, penicillin, tetracycline, gentamicin, cefixim, and ciprofloxacin. The accuracy of this test species identification with Müller Hinton Broth (MHB) and then standardized suspended in 0.5 McFarland was performed to compare all the bacteria colonies tested. When it ensures a perfect match for a sterile cotton swab employing 4 separate culture bacteria in a medium-sized medical Mueller Hinton (MHA), the disks and antibiotic disks

applied in sterile conditions have been cultivated concerning the distance of the medium Mueller Hinton agar plates of two centimeters from each other. Following incubation at 37ºC for 18 to 24 hours, the findings obtained were contrasted with sensitive and immune to the requirements of Laboratory standard [10].

**Preparing ethanol extract of sage plant:**

Agricultural and natural resource sage plant samples (leaves and stems) were prepared, and an electric crusher then did air-dried powder. Solvent ethanol was made from a wise ethanol extract from the plant 95%. Initially, 50 grams of powder were weighed and placed in the center and then in a soxhlet tank; then 250 ml of ethanol was cast into a soxhlet flask and extracted for eight hours. Throughout this period, the extract has been poured to dry at room temperature in a sterilized glass plate. The extract can be stored in a cooler under 40C before it is used [11].

**Antibacterial influence of ethanol extracting of Salvia based on well diffusion method:**

The solution with 10 percent DMSO was developed to assess the antimicrobial activity of a salvia (Sage) leaf and stem extract from 50, 100,150 mg/ml of ethanol extract. In the medium MHB, McFarland was prepared for 24-hour cultivation of all bacteria strains of Turbidity, equal to a norm 0.5. The suspension turbidity in the absorption spectrophotometer 0.8 is calculated for greater precision. Suspension was then cultivated using sterile swabs on the media of MHA in 4 directions. A sterile Pasteur pipette with a diameter of 6 mm to the produced Wells with a distance of 2.5 cm from the crop after 0.5 hours. Hundred µL have been individually added from each well with various ethanol extract concentrations. As a negative control, DMSO is 10% of the solution. The inhibition zone diameter around each well was measured using a rule after 24 h incubation at 37ºC. Experiments were replicated 3 times to verify the findings [12].

**Cell line culture:**

MCF-7 cells15 of human breast adenocarcinoma were collected from the American Type Culture Set (ATCC) and cultivated in DMEM medium supplemented by 10% fetal serum bovine, 1% sodium bicarbonate, 103 IU penicillin G and 100 µg streptomycin. In a CO2 incubator (5 percent) at 37EC, the MCF-7 cells were kept.

**MTT cytotoxicity assay:**

A procedure was carried out according to the instructions of the manufacturer. A 96 flat-well tile with a determined volume of Cells (1 TH104 to 1 TH106 CML) was cultivated. The plate was sterilized, gently stirred and incubated at 37EC, 5% carbon dioxide, for 24 hours. The medium was extracted after incubation by inserting 200 metric mL (25, 50 m, 100, 200 mg, 400 mg mLG1) in the wells from the 2-fold serial dilutants of Salvia Sp (Sage plant). At each concentration, triplicates and controls were performed. Plates have been incubated at 37EC, 5% CO2 in 24 hours. 10 mL of the MTT solution is applied to every well after exposure to the extract. Further incubation was done at 37EC, 5% CO2 for 4 hours. A medium was extracted thoroughly; 100 ml of solution was applied to the pot and incubated for 5 minutes. The absorption was measured with a 575 nm wavelength ELISA reader (Bio-rad, Germany). In order to quantify 16 IC 50, the optical density measurements were statistically analyzed.

Statistical analysis: The ANOVA method was carried out to determine whether group variance is significant. Mean±Standard Deviation (SD) data was expressed, while statistical value was taken using a version of GraphPad Prism 6.

## RESULTS

Antibiotic screen results were tabulated in Tables 1 and 2. Following studies accomplished in this study (Table), the leaves extracted by ethanol and stems of Sage at different concentrations such as 50, 100, and 150 mg/mL show it possesses antibacterial capacity against bacterial strains of multidrug-resistant P. aeruginosa, Klebsiella pneumonia, Staph. aureus, Providencia, Burkholderia, and E. coli; moreover, ethanol extract of Sage on multidrug-resistant Gram-positive Staphylococcus aureus had the major efficiency. Three bacteria, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae, demonstrated high sensitivity to ethanol extract from the leaves and stems of sage. All extracts exhibited dose following activity, with the intention of concentration. The MIC and MBC values of the ethanol extract of sage are tabulated in Table 1,2,3 against the mentioned bacteria. As the table exhibited, ethanol extract can restrain the expansion of bacteria like S. aureus, E. coli, P. aeroginosa and K. pneumonia.

| Antibiotic | Resistant S.aureus | Sensitive S.aureus | Sensitive E.coli | Resistant E.coli |
|---|---|---|---|---|
| **Erythromycin** | 90 | 10 | 5 | 95 |
| **Amoxicillin** | 34 | 66 | 33 | 67 |
| **Penicillin** | 80 | 20 | 9 | 91 |
| **Gentamicin** | 38 | 62 | 32 | 68 |

**Table1. Percentage of antibiotic sensitivity and resistance of *S.aureus* and *E.coli***

| Antibiotic | Resistant *P. aeruginosa* | Sensitive *P. aeruginosa* | Sensitive *Burkholderia* | Resistant *Burkholderia* |
|---|---|---|---|---|
| **Ampicillin** | 33 | 67 | 30 | 70 |
| **Penicillin** | 80 | 20 | 75 | 25 |
| **Gentamicin** | 53 | 47 | 49 | 51 |
| **Clindamycin** | 70 | 30 | 66 | 34 |

**Table 2. Percentage of antibiotic sensitivity and resistance of *P. aeruginosa* and *Burkholderia.***

| Antibiotic | Resistant *Klebsiella pneumonia* | Sensitive *Klebsiella pneumonia* | Sensitive *Providencia.* | Resistant *Providencia.* |
|---|---|---|---|---|
| **Erythromycin** | 8 | 92 | 18 | 82 |
| **Ampicillin** | 10 | 90 | 40 | 60 |
| **Cefixime** | 11 | 89 | 28 | 72 |
| **Gentamicin** | 46 | 54 | 40 | 60 |

**Table 3. Percentage of antibiotic sensitivity and resistance of *Klebsiella pneumonia* and *Providencia.***

| Bacteria | 50mg/ml | 100 mg/ml | 150 mg/ml | Control- | Control+ |
|---|---|---|---|---|---|
| ***Stph* .aureus** | 8 | 12 | 14 | ----- | 13 |
| ***Klebsiella pneumonia*** | 5.7 | 6.8 | 9,8 | ----- | 4.9 |
| ***Providencia*** | | | | ----- | |
| ***P. aeruginosa*** | 4.4 | 7,8 | 8.9 | ------ | 7.8 |
| ***Burkholderia*** | 4.2 | 7.3 | 8.1 | ------ | 6.9 |
| ***E.coli*** | 5 | 6 | 6.4 | ------ | 9 |

**Table 4. Antibacterial activity of sage ethanol extract against tested bacteria measured in millimeters.**

Besides, the inhibition zone was amplified by increasing the ethanol extract concentration ($p \leq 0.001$). These results exhibited that there was a considerable difference ($p \leq 0.001$) in terms of thoughtfulness to ethanol extract. Furthermore, the major sensitivity was paid attention in S. aureus, and the minor was noticed in K. pneumoniae. MTT assay was utilized to determine the cytotoxic influence of Sage extracts on MCF-7 cells: This test of 3-(dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was utilized to detect the cytotoxic influence of Sage extract on MCF-7 cells. This assay was fulfilled to measure the cell viability and inhibition rate by utilizing diverse concentrations of Sage extract at tumor cell lines.

The analysis data was done by µg mLG1, and log values of µg mLG1 were organized by a Graphpad Prism 6 utilizing log (Inhibitor) versus responsecurve. The efficient concentrations were picked according to the efficient $IC_{50}$ values. The capability of Cell

fixed. The Results recorded that exposure of MCF-7 cells to methanol extract in certain concentrations (0.5-3.00 µg mLG) for 24 hrs. displayed a reduction in cell viability in a dose-subject manner; moreover, the cell viability diminutive with raising the concentrations of methanolic extract. The minimum MCF-7 cell viability (40%) was determined at 2.5 µg mLG1. potent cytotoxic capacity with an $IC_{50}$ value of 2.5 µg mLG1. This effect proposes that the methanolic extract is cytotoxic against MCF-7 cells, as motioned by the MTT assay. The reduction of the cell count was dose following as well the major considerable reduction appeared ($p<0.0001$) when enforcing a high dose (3.00 µg mLG1) of the extract [13].

| | Nonlinear fit | A | B | C |
|---|---|---|---|---|
| | | | | |
| | | MCF-7 | WRL68 | Global (shared) |
| | | | | |
| | | Y | Y | Y |
| 1 | Comparison of Fits | | | |
| 2 | Null hypothesis | | | One curve for all data sets |
| 3 | Alternative hypothesis | | | Different curves for each data set |
| 4 | P value | | | < 0.0001 |
| 5 | Conclusion (alpha = 0.05) | | | Reject null hypothesis |
| 6 | Preferred model | | | Different curves for each data set |
| 7 | F (DFn, DFd) | | | 167.1 (4,34) |
| 8 | | | | |
| 9 | Different curves for each data set | | | |
| 10 | Best-fit values | | | |
| 11 | Bottom | 39.07 | 81.25 | |
| 12 | Top | 99.09 | 95.98 | |
| 13 | LogIC50 | 1.801 | 2.216 | |
| 14 | HillSlope | -1.501 | -3.672 | |
| 15 | IC50 | 63.18 | 164.5 | |
| 16 | Span | 60.02 | 14.73 | |
| 17 | Std. Error | | | |
| 18 | Bottom | 4.560 | 1.313 | |
| 19 | Top | 3.347 | 0.4977 | |
| 20 | LogIC50 | 0.06203 | 0.04297 | |
| 21 | HillSlope | 0.3519 | 1.055 | |
| 22 | Span | 6.943 | 1.468 | |
| 23 | 95% Confidence Intervals | | | |
| 24 | Bottom | 29.45 to 48.69 | 78.48 to 84.02 | |
| 25 | Top | 92.03 to 106.2 | 94.93 to 97.03 | |
| 26 | LogIC50 | 1.670 to 1.931 | 2.125 to 2.307 | |
| 27 | HillSlope | -2.244 to -0.7590 | -5.897 to -1.44 | |
| 28 | IC50 | 46.74 to 85.40 | 133.5 to 202.6 | |
| 29 | Span | 45.38 to 74.67 | 11.64 to | |

| | | | | |
|---|---|---|---|---|
| | | | 17.83 | |
| **30** | Goodness of Fit | | | |
| **31** | Degrees of Freedom | 17 | 17 | |
| **32** | R square | 0.9658 | 0.9312 | |
| **33** | Absolute Sum of Squares | 315.2 | 45.33 | |
| **34** | Sy.x | 4.306 | 1.633 | |
| **35** | | | | |
| **36** | One curve for all data sets | | | |
| **37** | Best-fit values | | | |
| **38** | Bottom | 54.80 | 54.80 | 54.80 |
| **39** | Top | 98.30 | 98.30 | 98.30 |
| **40** | LogIC50 | 1.996 | 1.996 | 1.996 |
| **41** | HillSlope | -1.208 | -1.208 | -1.208 |
| **42** | IC50 | 99.17 | 99.17 | 99.17 |
| **43** | Span | 43.50 | 43.50 | 43.50 |
| **44** | Std. Error | | | |
| **45** | Bottom | 23.51 | 23.51 | 23.51 |
| | | | | |
| | **Nonlin fit** | **A** | **B** | **C** |
| | | | | |
| | | **MCF-7** | **WRL68** | Global (shared) |
| | | | | |
| | | **Y** | **Y** | **Y** |
| **46** | Top | 8.987 | 8.987 | 8.987 |
| **47** | LogIC50 | 0.4087 | 0.4087 | 0.4087 |
| **48** | HillSlope | 1.272 | 1.272 | 1.272 |
| **49** | Span | 30.22 | 30.22 | 30.22 |
| **50** | 95% Confidence Intervals | | | |
| **51** | Bottom | 7.210 to 102.4 | 7.210 to 102.4 | 7.210 to 102.4 |
| **52** | Top | 80.11 to 116.5 | 80.11 to 116.5 | 80.11 to 116.5 |
| **53** | LogIC50 | 1.169 to 2.824 | 1.169 to 2.824 | 1.169 to 2.824 |
| **54** | HillSlope | -3.783 to 1.366 | -3.783 to 1.366 | -3.783 to 1.366 |
| **55** | IC50 | 14.76 to 666.5 | 14.76 to 666.5 | 14.76 to 666.5 |
| **56** | Span | -17.68 to 104.7 | -17.68 to 104.7 | -17.68 to 104.7 |
| **57** | Goodness of Fit | | | |
| **58** | Degrees of Freedom | | | 38 |

| | | | | |
|---|---|---|---|---|
| **59** | R square | 0.5816 | -4.444 | 0.4772 |
| **60** | Absolute Sum of Squares | 3860 | 3588 | 7448 |
| **61** | Sy.x | | | 14.00 |
| **62** | Constraints | | | |
| **63** | Bottom | Bottom is shared | Bottom is shar | |
| **64** | Top | Top is shared | Top is shared | |
| **65** | LogIC50 | LogIC50 is shared | LogIC50 is sha | |
| **66** | HillSlope | HillSlope is shared | HillSlope is sha | |
| **67** | | | | |
| **68** | Number of points | | | |
| **69** | Analyzed | 21 | 21 | |

| **Row stats** | | **X** | | **A** | | | **B** | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | X Title | | **MCF-7** | | | **WRL68** | |
| | | | | | | | | |
| | | | | | | | | |
| | | **X** | **Mean** | **SD** | **N** | **Mean** | **SD** | **N** |
| | | | | | | | | |
| **1** | | 400.000 | 40.124 | 4.750 | 3 | 81.790 | 3.098 | 3 |
| | | | | | | | | |
| **2** | | 200.000 | 52.701 | 1.124 | 3 | 86.073 | 2.147 | 3 |
| | | | | | | | | |
| **3** | | 100.000 | 56.674 | 2.788 | 3 | 93.943 | 0.594 | 3 |
| | | | | | | | | |
| **4** | | 50.000 | 73.881 | 4.386 | 3 | 95.833 | 1.029 | 3 |
| | | | | | | | | |
| **5** | | 25.000 | 87.963 | 6.928 | 3 | 95.409 | 0.707 | 3 |
| | | | | | | | | |
| **6** | | 12.500 | 95.756 | 0.267 | 3 | 96.721 | 2.233 | 3 |
| | | | | | | | | |
| **7** | | 6.250 | 95.718 | 0.612 | 3 | 95.756 | 0.438 | 3 |
| | | | | | | | | |

**Table 5: The cancer cell results.**

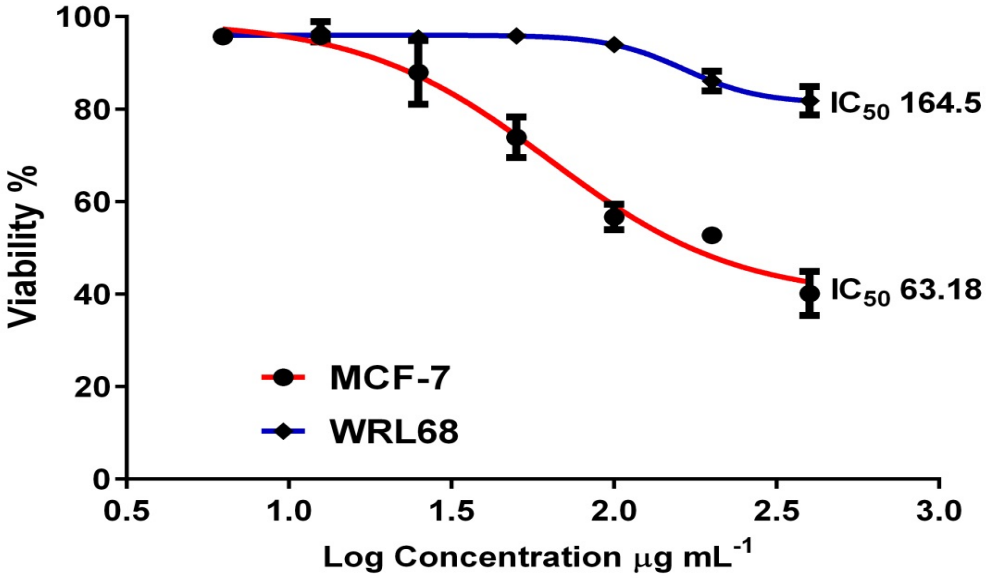


**Figure 1: The inhibition of cancer cell growth**

## DISCUSSION

The results show that the ethanol extract of sage at 50, 100 and 400 mg/mL has prevented gram-negative and gram-positive bacteria from growth. The study, therefore, demonstrates the antibacterial special effects of Gram-negative and Gram-positive multi-drug resistant bacteria on the medicinal herb. The inhibition zone also increases with the incremental rise in concentration. S was found to be the most sensitive. K has been seen in Aureus and the least. The findings show the inhibitory effects of Sage's ethanol extract on MIC = 18.75 mg/mL for S. (minimum inhibitory concentration) MIC = 26.56 mg/mL for E. aureus. Coli, for P. aeruginosa with MIC=33.75 mg/ml and for K with MIC=31.25 mg/mL. Air conditioning. Pneumoniae. There is a partial distinction between these findings and related studies as regards the extracting method and avoiding the use of extraordinary temperatures to avoid the destruction of herbal compounds. Sabia essential oil had antibacterial activity against S. Balsa, L. M.et al. Aura and E. Aura. MIC=11 mm for S and coli. MIC=23mm for E and aureus. Mad. This research has shown that our findings show a higher effect than ethanol extract on the essential oil [14].

As reported in 2019, Sage ethanol extracts have a gram-negative influence in contradiction of normal strains of gram-positive bacteria (S. aureus ATCC6538, B. subtilis, ATCC6633) and gram-negative bacteria and have an inhibitive effect of sage ethanol extract with S. coli As ATCC25922, P. aeraoginosa As, As and ATCC13076 with MIC = 10 mg/mL. MIC=6 mg/mL for B, aureus. MIC=60 mg/mL in E subtilis. Coli, MIC=60 mg/mL, and MIC=50 mg/mL for S. For P. aeroginosa. enteritidis that had an important. Present studies discrepancies. In 2004, Lai and others wrote. Bacillus cereus, Staphylococcus aureus and Vibrio Sage ethanol extract Bacteria. Parahaemolyticus is successful, and wise ethanol extract has multidrug-resistant S antibacterial activity in this research. Core, E. Coli, P. and K. Colli. Lung disease [15]. Sage's essential oil and fractions have specified a substantial antibacterial influence against S. B and Aureus. Subtilis. Minimum concentrations of inhibitors for S were 1.25-2.5 μL/mL. 0.15-2.5 μL/mL and B for aureus [16]. This research has shown that the sage ethanol extract

is antibacterial to selected multi-drug resistant bacteria. In addition to ethanol extract, the essential oil was more efficient [17]. Mehrabi, A. et al. tested sage essential oil with Gram-positive (S. aureus and Streptococcus D) and Gram-negative bacteria (E. coli, S. typhi and P. aeruginosa) and antibacterial against Gram-positive bacteria with similar results. When we compared these findings, the impact of basic oil is greater than ethanol extracting [18].

The request of Canalso is essential in treating disease in plant extracts with established antimicrobial properties. Studies in numerous countries have been performed in recent years to show the productivity of plants. Many plants use antimicrobial treatment since the compounds of the secondary metabolism of plants are synthesized. Many experiments have demonstrated the biological activity of Lamiaceae extracts, such as sage, against bacteria and yeasts. Salvia Officinalis ethanol extract can avert multi-drug-resistant bacteria [19]

S. Core, E. Aeruginosa, P. coli and K. Air conditioning. Pneumoniae. Since antibiotic resistance is rising in many countries. These effects, in particular the high impact [20].

Bacteria can have significant concentrations of ethanol extract. However, more and more comprehensive studies and effective and standardized results are required for the clinical application of this plant. Instead of inert and inefficient antimicrobial drugs currently in use, these plants may use as an alternative.

## CONCLUSION

It was concluded that the plant extract of sage is a source of many compounds with biological activities that can be potential alternatives for preparing herbal medicines that have efficacy on some microorganisms and cancer cells. This research aims to examine the antibacterial performance of sage ethane extraction using well-dissemination and multi-drug resistant bacteria.

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