World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

# Research on the antimicrobial activity of the plant species *Salvia officinalis*

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World Journal of Advanced Research and Reviews, 2025, 26(03), 1604-1611

Publication history: Received on 26 April 2025; revised on 11 June 2025; accepted on 13 June 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.3.2309

## Abstract

The process of minimizing the manifestation of storage diseases of fruits by using treatments with biological products is a basic premise in the development of organic agriculture. On 01.03.2022, inoculations were made on a culture medium established using a proprietary recipe that supports the growth and development of any living organism. The culture medium represents the physical and chemical support necessary for the growth and development of any living organism, including pathogenic ones. It also must respond, through its composition, to the nutritional and hormonal requirements of the organism in question. The experiment consisted of a micro dilution of sage essential oil (*Salvia officinalis)* in the composition of the biopreparations, made according to a proprietary recipe, that were used to determine the minimum inhibitory concentration (MIC). MBC corresponds to the minimum concentration of sample that can kill the microorganism. The same micro dilution experiment derived from the MIC determination was used.

**Keywords:** *Salvia officinalis*; Biological agriculture; Pathogen; Biopreparation; Minimum inhibitory concentration

## 1. Introduction

Common sage (*Salvia* spp., the most common species being *Salvia officinalis)* is an important medicinal and aromatic plant due to its bioactive components, by-products of its metabolism [1,3]. These components are mainly phenolic compounds, terpenoids, polyphenols and flavonoids. Numerous studies have identified its important role in combating oxidative stress in cells and organisms, along with its anticancer, antimicrobial and anti-inflammatory roles. [2]. There are numerous methods to measure the antioxidant activity of sage phenolic components, usually based on the scavenging of free radicals from free radical species, such as 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and the determination of the absorbance of the reduced product by a photometric assay. In addition, there are several *in vitro* and *in vivo* studies determining the protection of sage extracts in cultured cells or animals, respectively, after the induction of oxidative stress. In this review, the results of currently available studies revealing the significant role of sage bioactive compounds as antioxidant compounds, as well as the variety of methods used, were critically analyzed and discussed. [4, 5, 6, 7]

Experimental evidence suggests that most plants and spices possess a wide range of biological and pharmacological activities that can protect tissues against O2-induced damage. The objectives of the present study were: first, to determine the effects of plant extracts on the viability, membrane integrity, antioxidant status, and DNA integrity of Caco-2 cells, and second, to investigate the cytoprotective and genoprotective effects of these plant extracts against oxidative stress in Caco-2 cells. [8, 9, 10]

*Salvia officinalis* L., commonly known as sage, is extremely rich in polyphenolic compounds, which are believed to be responsible for its abundant antioxidant potential. *Salvia* consists of a complex mixture of monoterpenes, diterpenes, sesquiterpenes, flavonoids and phenolic acids. Together with other phytoconstituents such as cineole, borneol,

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rosmarinic acid, chlorogenic acid, salvianolic acid or vitamins C and E, sage represents the best way to protect individuals against different types of negative effects caused by xenobiotics [11, 12]. Salvia-based products have been confirmed to exhibit beneficial anti-inflammatory, antihyperglycemic, spasmolytic, antiseptic or hepatoprotective activities. In addition, some experimental studies indicate that molecules contained in *Salvia* eliminate free radicals, thus minimizing the harmful effects of oxidative stress [13, 14]. The experiments aimed to determine whether the use of Sage biopreparations, with known composition, which show positive effects on antimicrobial activity on pathogens that cause storage diseases during the fruit storage period.

## 2. Material and methods

The plant material consists of samples of *Salvia officinalis* plants from SCDL Buzău, essential oils were extracted from plants harvested in different phases of vegetation (vegetative stage, beginning of flowering stage, full flowering stage).

The antimicrobial activities of sage essential oils, extracted at three developmental stages of the plants, were performed using the disk diffusion technique against three bacterial strains *Monilinia fructigena, Venturia inaequalis* (Figures 1 and 2).

Thus, three recipes for biopreparations were created that included essential oils of sage, fennel, and coriander extracted at different stages of the plant's development.

### 2.1. Operational method

The primary screening of the antimicrobial activity of the studied samples was evaluated by the disk diffusion method as previously published. Briefly, the culture suspension of each species was inoculated into the optimal culture medium [15]. Subsequently, sterile paper discs with a diameter of 6 mm, soaked with 10 µL of essential oil (mixed with 5% DMSO) from the three phenological stages, were deposited on each plate. Chloramphenicol (30 µg) was used as positive control for bacteria and nystatin (100 IU) was used as a positive control for yeast and fungi, while DMSO (10 µl; 5%) was used as a negative control. The plates were incubated under the following growth conditions; 37 °C for 24 hours, 25 °C for 48 hours and 25 °C for 72 hours, for bacteria, yeast and fungi, respectively. After incubation, inhibitory diameters were measured in millimeters and results are expressed as means ± standard deviation of three replicates.

The micro dilution experiment of the mixture was used to determine the minimum inhibitory concentration (MIC) as previously reported. The MIC corresponds to the minimum concentration of sample that can kill the microorganism. The same micro dilution experiment derived from the MIC determination was used. After incubation, 10 µL of each tube that did not show visible growth was subculture onto Tryptone Soy Agar (Biokar, Beauvais, France) and incubated at 37°C for 24 hours, and the lowest concentration that did not show any growth on the medium was considered as the minimum bactericidal concentration (MIC).


**Figures 1 and 2** Pathogens *Monilinia fructigena* and *Venturia inaequalis* (own source)

Thus, for *Salvia officinalis,* three solutions were prepared, each from the same base to which different essential oils extracted from different stages of plant vegetation were added, extracted as follows:

SO1 – vegetative stage, SO2- Beginning of flowering stage, SO3- full flowering stage.

The basic formula used was made of: Tensida consistency 35%, Bio lavender base 48.8%, Sage water 15%, sage essential oil 15 drops, cosgard 6 drops for a total volume of 50 ml.

Tests were performed to highlight the possible bactericidal and/or bacteriostatic effects of the essential oils, and the results were recorded in tables 1 and 4.

The data resulting from the measurements were statistically analyzed using ANOVA software.

## 3. Results and discussion

**Table 1** Results on the MIC and MBC of essential oils extracted *from Salvia officinalis* in percentages (v/v) in three developmental stages of plant development against the pathogen *Monilinia fructigena (Brown rot*).

| ID no. | *Monilinia fructigena* | CIM and CBM results of sage essential oils % (v/v) | | |
|---|---|---|---|---|
| | | R1 | R2 | R3 |
| 1 | CIM | 1 | 0.50 | 0.50 |
| 2 | CBM | 2 | 1 | 0.50 |

Where R1 is SO1-Vegetative stage, R2 is SO2-Beginning of flowering stage and R3 is SO3-Full flowering stage MIC - minimum inhibitory concentration and MBC - minimum bactericidal concentration.

The results presented in Table 1 regarding the MIC and CBM of the essential oils extracted from *Salvia officinalis* in percentages (v/v) from sage harvested at all three stages of plant development (vegetative stage, beginning of flowering stage, full flowering stage) and applied against the pathogen *Monilinia fructigena (Brown rot),* are expressed as the average zone of inhibition of the pathogen compared to the fungicide boscalid 4 (μg/ml). The results were very significant, with the oils being antibacterial against the tested pathogen *Monilinia fructigena* compared to this fungicide ($p \leq 0.05$), this may also be due to the fact that the essential oil was in pure form and the fungicide boscalid was at a concentration of 30 μg/disc.

**Table 2** ANOVA results on the average MIC and MBC values of essential oils extracted *from Salvia officinalis* in percentages (v / v) in three developmental stages of the plant (vegetative stage, beginning of the flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena (Brown rot*).

| ID. no | *Monilinia fructigena* | Average value | Average report% | Average difference | General average |
|---|---|---|---|---|---|
| 1 | CIM | 0.67 | 72.73 | - 0.25 | 0.92 |
| 2 | CBM | 1.17 | 127.27 | 0.25 | |

In table 2 we have ANOVA results regarding the average MIC and MBC values of the essential oils extracted from *Salvia officinalis* in percentages (v / v) in three stages of development of plants (vegetative stage, beginning of the flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena (Brown mold)* with the general values: MIC average value= 0.67 – indicates that a relatively low concentration of essential oil is sufficient to inhibit the growth of *Monilinia fructigena.* This shows a good antimicrobial activity and MBC average value = 1.17 – represents the concentration required to destroy the microorganism completely.

It is higher than the MIC, which is normal, but still a fairly low value, suggesting good effectiveness as a bactericidal agent.

The overall average (MIC and MBC) =0.92 – reflects a balance between the inhibitory and bactericidal effect, indicating that sage essential oils can be considered promising alternatives to classic fungicides.

**Table 3** ANOVA test results on the MIC and MBC of essential oils extracted *from Salvia officinalis* in percentages (v / v) in three developmental stages of the plant (vegetative stage, beginning of the flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena (Brown rot*).

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***P-value*** | ***F crit*** |
| Between Groups | 0.375 | 1 | 0.375 | 1.125 | 0.348641 | 7.708647 |
| Within Groups | 1.3333 | 4 | 0.33333 | | | |
| Total | 1.7083 | 5 | | | | |

- H $_{0}$ - (null hypothesis): all group means are equal.
- H $_{1}$ – (alternative hypothesis): at least one group has a different mean

In Table 1 are the results of the ANOVA test on the MIC and MBC of the essential oils extracted *from Salvia officinalis* in percentages (v / v) in three in three developmental stages of the plant (vegetative stage, beginning of the flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena (Brown rot*), since the calculated F is smaller than the critical F 1.12 < 7.71 in this case there are no statistically significant differences between the three stages. Any difference observed between their means would be due to chance.

In this case calculated F < critical F – there is insufficient evidence to reject the null hypothesis $H_0$, there are no significant differences between the compared means.

The calculated factor 1.12 is less than the DL 1.3086, then the differences between the essential oil treatments in the three phases are not statistically significant at the chosen significance level.

Conclusion: There are no significant differences between the treatments in the three phases analyzed regarding the effect of sage essential oils against the pathogen *Monilinia fructigena – The brown grebe.*

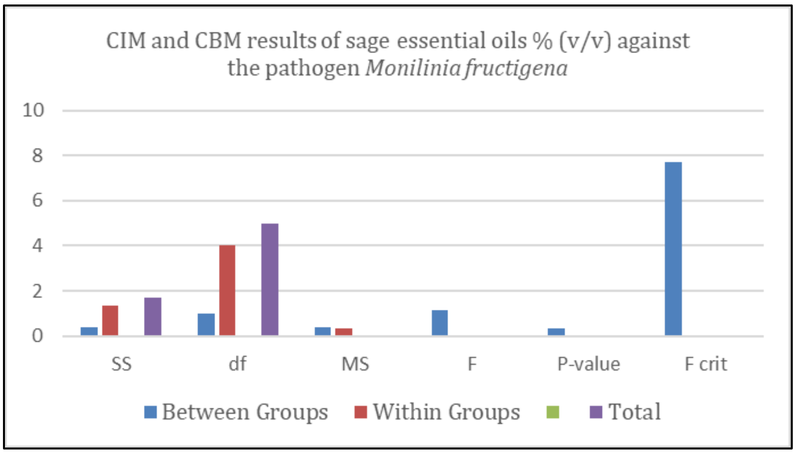


**Figure 3** Figure representation of the results regarding the MIC and MBC of the essential oils extracted from *Salvia officinalis* in percentages (v/v) in three stages of development of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena (Brown rot*)

In figure 3 is the graphical representation of the results regarding the MIC and MBC of the essential oils extracted from *Salvia officinalis* in percentages (v/v) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Monilinia fructigena (Brown rot*) where the difference limit DL=1.3086 is the minimum threshold above which a difference between two environments is considered significant.

The overall mean of 0.92 is lower than DL5=1.31, below the significant limit, which indicates that the differences between means or variations in the data are not statistically significant, they do not exceed the established significance threshold.

The correlation ratio = 0.4685 is a correlation level that indicates a **moderate, positive linear relationship** between two variables, but not strong enough.

**Table 4** Results regarding the MIC and MBC of essential oils extracted *from Salvia officinalis* in percentages (*v* / *v*) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis* (which causes the scab disease)

| ID. no. | *Venturia inaequalis* | CIM and CBM results of sage essential oils % (v/v) | | |
|---|---|---|---|---|
| | | R1 | R2 | R3 |
| 1 | CIM | 1 | 0.50 | 0.50 |
| 2 | CBM | 1 | 1 | 0.50 |

Where

- R1 is SO1-Vegetative stage,
- R2 is SO2-Beginning of flowering stage and R3 is SO3-Full flowering stage;
- MIC - minimum inhibitory concentration and MBC - minimum bactericidal concentration.

The results in Table 4 regarding the MIC and MBC of the essential oils extracted *from Salvia officinalis* in percentages (*v*/*v*) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis* (the disease caused by – scab), obtained in the case of *Salvia officinalis* are expressed as the average zone of inhibition of the three stages of development compared to the fungicide Score 4 µg/ml and were highly significant as antibacterial against *Venturia inaequalis* tested in comparison to this fungicide ( $p \leq 0.05$).

**Table 5** Results of average values regarding MIC and MBC of essential oils extracted *from Salvia officinalis* in percentages (*v*/*v*) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis*

| ID. no. | *Venturia inaequalis* | Average value | Average report% | Average difference | General average |
|---|---|---|---|---|---|
| 1 | CIM | 0.67 | 88.89 | - 0.08 | 0.75 |
| 2 | CBM | 0.83 | 111.11 | 0.08 | |

In Table 5 we have the results of the average values regarding the MIC and MBC of the essential oils extracted from *Salvia officinalis* in percentages (*v* / *v*) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis.*

Where MIC = 0.67 – indicates that a relatively low concentration of essential oil is sufficient to inhibit the growth *of Venturia inaequalis.* Good inhibitory activity and MBC = 0.83 – slightly weaker, but still effective bactericidal activity.

The MBC/MIC ratio is 1.24 – this ratio suggests effective bactericidal activity, not just bacteriostatic.

**Table 6** ANOVA test results on the MIC and MBC of essential oils extracted *from Salvia officinalis* in percentage (*v* / *v*) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis*

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***P-value*** | ***F crit*** |
| Between Groups | 0.041667 | 1 | 0.041667 | 0.5 | 0.518519 | 7.708647 |
| Within Groups | 0.333333 | 4 | 0.083333 | | | |
| Total | 0.375 | 5 | | | | |

- Hypotheses: H $_0$ - (null hypothesis): all group means are equal and
- H $_1$ - (alternative hypothesis): at least one group has a different mean

In Table 6 are the results of the ANOVA test on the MIC and MBC of the essential oils extracted from *Salvia officinalis* in percentage (*v / v*) in three developmental stages of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis.* Since the calculated F is smaller than the critical F 0.500 < 7.71 in this case there are no statistically significant differences between the three stages. Any difference observed between their means would be due to chance.

In this case calculated F < critical F – there is insufficient evidence to reject the null hypothesis $H_0$, there are no significant differences between the compared means.

The calculated factor 0.05 is less than the DL 0.6543, then the differences between the essential oil treatments in the three phases are not statistically significant at the chosen significance level.

Conclusion: There are no significant differences between the treatments with sage essential oil extracted in all three vegetation phases of plants (vegetative stage, beginning of flowering stage, full flowering stage), analyzed regarding the effect of sage essential oils on the pathogen *Venturia inaequalis.*

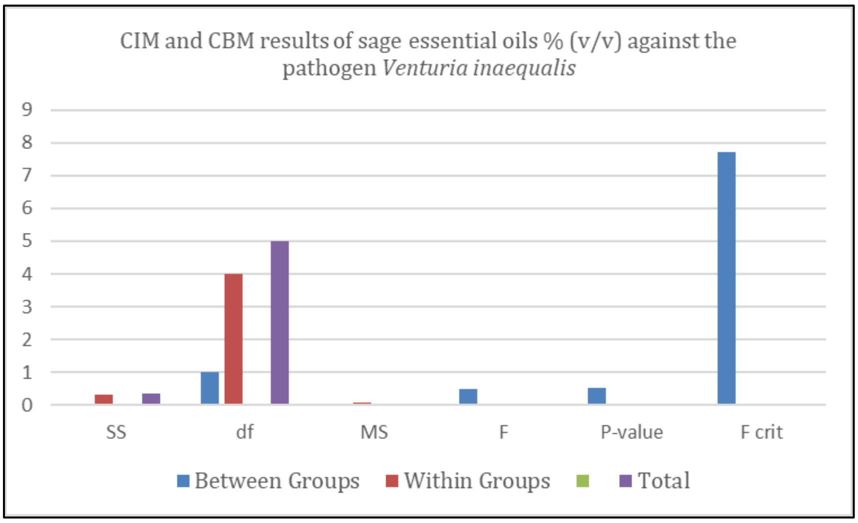


**Figure 4** Graphical representation of the results regarding the MIC and MBC of the essential oils extracted from *Salvia officinalis* in percentages (v / v) in three stages of development of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis*

In figure 4 we have the graphical representation of the results regarding the MIC and MBC of the essential oils extracted from *Salvia officinalis* in percentages (v / v) in three stages of development of the plant (vegetative stage, beginning of flowering stage, full flowering stage) used against the pathogen *Venturia inaequalis* where difference in the DL boundary=0.6543 - The differences between the confidence interval boundaries were 0.6543 DL, reflecting a moderate degree of precision in the effect estimate.

The values obtained for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sage essential oils, extracted at different phenological phases of plants (vegetative stage, beginning of flowering stage, full flowering stage), showed an overall average of 0.75mg/mL. This value suggests a moderate antimicrobial activity of the tested compound.

The correlation ratio calculated between MIC and MBC = 0.33 indicating a weak positive connection which implies that the values of the two parameters tend to vary in the same direction, but without a close statistical relationship. Regarding the differences between the values recorded in the three phenological phases, they did not exceed the significant threshold established by the difference in significance limit (DL=0.65), which confirms that the observed differences are not statistically significant.

Therefore, the development vegetative phase of the *Salvia officinalis* plant did not significantly influence the antibacterial activity of sage essential oils under the experimental conditions tested.

The microbial activity of sage oil is relatively constant, with no significant differences between phases. There is a weak thread of correlation between MIC and MBC, but not one strong enough for predictive conclusions.

The essential oil of *Salvia officinalis* holds promising antimicrobial efficacy against *Venturia inaequalis*.

## 4. Conclusion

The overall average (MIC and MBC) =0.92 – reflects a balance between the inhibitory and bactericidal effect, indicating that sage essential oils can be considered promising alternatives to classic fungicides.

There are no significant differences between treatments with sage essential oil in all the three vegetative phases analyzed (vegetative stage, beginning of flowering stage, full flowering stage), regarding the effect of sage essential oils against the pathogen *Venturia inaequalis*.

Therefore, the development of the vegetative phase of the *Salvia officinalis* plant did not significantly influence the antibacterial activity of sage essential oils under the experimental conditions tested.

The microbial activity of sage oil is relatively constant, with no significant differences between phases. There is a weak thread of correlation between MIC and MBC, but not one strong enough for predictive conclusions.

The essential oil of *Salvia officinalis* holds promising antimicrobial efficacy against *Venturia inaequalis.* From a statistical and practical point of view, any stage can be chosen for extraction, without loss of anti-fungal efficiency.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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