**JSMARTech**
Journal of Smart Bioprospecting and Technology **p-ISSN**: 2686-0805 **e-ISSN**: 2714-7894
October 2023, VOL 04 NO 02 **DOI**:https://doi.org/10.21776/ub.jsmartech.2023.004.02.67

# Antimicrobial Activity of Tobacco Flower Extract (*Nicotiana tabacum* L.) in Various Solvent

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**Submission**: 8 October 2023; **Revised**: 25 October 2023; **Accepted**: 26 October 2023

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***ABSTRACT.*** *Tobacco plants (Nicotiana tabacum L.) are known to contain secondary metabolites that function as antimicrobials. But thus far only the leaves, roots, and stems have been utilized. The research aimed to determine antimicrobial activity of tobacco flower extract in various solvent. The tobacco flowers were extracted used ethanol, methanol, and N-Hexane solvents. The extracts were tested for antimicrobial activity against Staphylococcus aureus and Candida albicans by dilution method. The results showed that ethanol and methanol extracts of tobacco flowers had a Minimum Inhibitory Concentration (MIC) value of 32% and a Minimum Bactericidal Concentration (MBC) of 64% against S. aureus and C. albicans. As for the N-Hexane extract of tobacco flowers, it has MIC value of 64% and MBC of 64% against C. albicans. In conclusion, tobacco flower extract in ethanol, methanol, and N-hexane solvent have potential functions as antimicrobial agents.*

***Keywords:*** *antimicrobial, solvent, tobacco flower extract*

## INTRODUCTION

Plants are one of the natural materials that can be utilized for health. Medicinal plants contain secondary metabolite compounds that can function as therapeutic agents or as pharmaceutical ingredients. Secondary metabolites can function as antimicrobials, anti-inflammatories, antioxidants, and others. Each part of the plant contains secondary metabolites and has different antimicrobial abilities. Plant extraction is one of the ways taken to obtain these secondary metabolites.

*Nicotiana tabacum* commonly referred to as tobacco, is a herbaceous plant that belongs to the Solanaceae group. In some previous studies, it has been mentioned that tobacco plants contain secondary metabolites including cembranoids, flavonoids, alkaloids, and terpenoids [1][2]. Ether and ethanol extracts of tobacco leaves and seeds are known to have antibacterial activity against the *Staphylococcus* group. Methanol extract of tobacco leaves is also known to have inhibitory activity against *Candida albicans* [3]. The leaves, roots, and stems have been widely studied regarding their content and benefits, but the flowers have not been explored yet, especially their antimicrobial abilities. Thus far, flowers are only as waste that is not utilized. Previous research reported that tobacco flower extract has greater antimicrobial activity than its leaf extract [4].

One of the targets for antimicrobial development is *Staphylococcus aureus*, which is a major human pathogen and cause of various infections such as endocarditis, meningitis, and lung infections. The increase in *Methicillin Resistant Staphylococcus aureus* (MRSA) events has triggered alternative antimicrobial materials from natural materials [5]. Another target is *Candida albicans*, which is actually a normal flora in the body. However, in unhealthy immune system conditions, there will be excessive growth and can trigger infection [6].

Extraction solvents have an important role in the extraction of secondary metabolite compounds from plants. The solubility of secondary metabolite compounds will be influenced by the solubility of the solvent used [7]. Polar solvents that are usually used are methanol and ethanol, while the non-polar solvent used is N-Hexane. This study aims to determine the tobacco flower extract with various extraction solvents against antimicrobial activity.

## RESEARCH METHODS

### Sample Preparation

Tobacco flowers (*Nicotiana tabacum* L.) were obtained from Probolinggo, East Java. Tobacco flowers were washed thoroughly with running water, drained, dried at 45 ºC to obtain dry simplicia. The dried simplicia was pulverized into powder and sieved using a 40 mesh sieve [4][8].

### Extraction Tobacco Flowers

Tobacco flower simplicia powder was extracted using maceration method with methanol, ethanol, and N-hexane solvents (1:10) [8]. The extract was filtered using filter paper and concentrated using a rotary evaporator and evaporated in a waterbath to obtain a liquid extract of tobacco flower.

### Antimicrobial Activity Test

Antimicrobial activity testing uses the dilution method. The dilution method is used to determine the value of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Dilution is divided into liquid dilution and solid dilution [9]. The microbes used in this study were *Staphylococcus aureus* and *Candida albicans*. For the liquid dilution procedure, 1 mL of microbial suspension was included in 8 mL of Nutrient Broth media (for *S. aureus*) and Sabouraud Dextrose Broth media (for *C. albicans*), then 1 mL of liquid tobacco flower extract with concentrations of 2%, 4%, 8%, 16%, 32%, and 64%. The solution was then incubated at 37 ºC for 24 hours for *S. aureus* and 48 hours for *C. albicans* aerobically [10]. The turbidity of the solution was measured using UV-Vis spectroscopy at a wavelength of 580 nm. The MIC was determined by observing the turbidity and clarity of each incubated test media compared to the media control solution.

The liquid dilution solution was carried out the next procedure, namely solid dilution by taking 0.1 mL and placing it in a Petri dish, then adding Mueller Hinton Agar (MHA) media (for *S. aureus*) and Sabouraud Dextrose Agar (SDA) media (for *C. albicans*) with the pour plate method. The cultures were incubated at 37 ºC for 24 hours for *S. aureus* and 48 hours for *C. albicans* [10]. The results of the solid dilution were counted the number of microbial that grow using a colony counter.

## RESULTS AND DISCUSSIONS

Antimicrobial activity was tested using liquid and solid dilution methods. The dilution method used to determine the potential of a compound for antimicrobial activity by MIC and MBC. The MIC value is indicated from the lowest concentration of extract with microbes that give a clear solution color (no microbial growth) [9][11][12]. Figure 1 shows that the 32% and 64% extract concentrations began to show clarity that resembled the media control. To confirm the clarity, absorbance measurements were made to obtain quantitative values (Table 1).

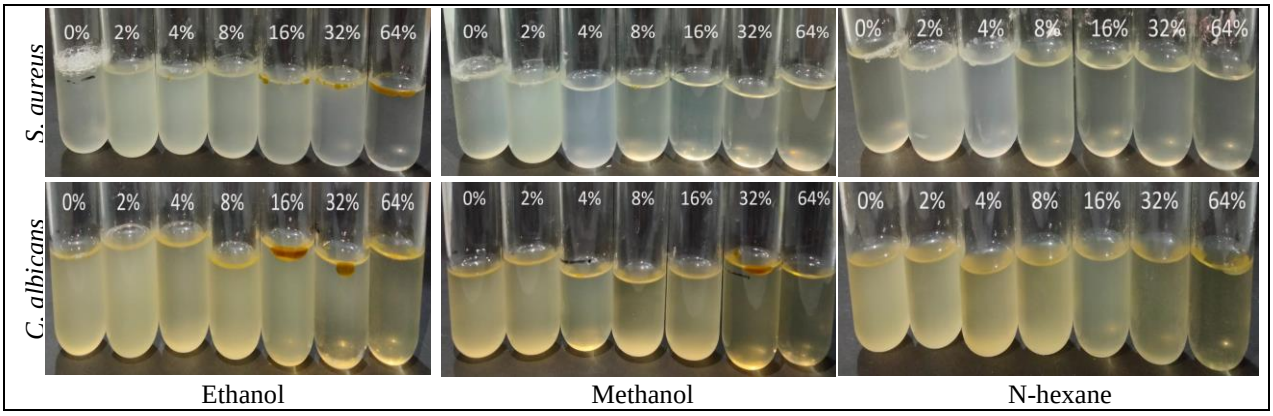


**Figure 1.** MIC Test Extract of Tobacco Flower against *S.aureus* and *C. albicans*

Tables 1 shows that the MIC value in ethanol and methanol extracts of tobacco flowers is at a concentration of 32%. While the MIC value in the N-Hexane extract is at a concentration of 64%. This demonstrates that antimicrobial compounds in tobacco flowers are more easily extracted using ethanol and methanol than n-hexane. solvent polarity affects the yield of extracts obtained [13] The solubility of the active compounds in the extraction solvent can result in different antimicrobial effects [14]. Tobacco flower extracts with three different types of solvents are able to inhibit the growth of both microbes, *S. aureus* and *C. albicans*.

Several components in tobacco plants such as polyphenols, terpenes, phytoalexins, and polysaccharides have antimicrobial activity. Previous research also mentioned that ethanol and methanol extracts from leaves and flowers were able to inhibit the growth of bacteria *S. aureus, E. coli*, and *B. subtilis*. While N-Hexane extracts

from tobacco roots are also able to inhibit the growth of *S. aureus*, *E. coli*, *C. albicans*, and *P. aeruginosa* [15][16].

From the results of the clear solution, solid diffusion was carried out by means of the results of the liquid dilution inoculated on petri dishes by the pour plate method using MHA media for *S. aureus* and SDA media for *C. albicans*. The MHA media has been recommended by the FDA and WHO for antibacterial tests, especially aerobic and facultative anaerobic bacteria for food and clinical materials [17]. Meanwhile, SDA media is a selective media for fungi that has an acidic pH of 5.6 so that it cannot be overgrown by other contaminant fungi. This media can be used to distinguish *C. albicans* from other types of fungi [6]. The MBC value is found at a concentration of 64% characterized by no microbial growth (Table 2). Antibacterial substances are substances that can interfere with the growth or metabolism of bacteria. Based on its activity, it can be divided into two types, namely bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria). This activity depends on the concentration and type of antibacterial material. The higher the concentration, the stronger the killing power of the substance against bacteria [12][18]. In addition, it is also influenced by the type of bacteria inhibited and the sensitivity of bacteria to inhibiting substances [19][20].

Table 2 shows that ethanol extract and methanol extract can kill *S. aureus* bacteria at a concentration of 64%, while the N-Hexane extract has not been able to kill bacteria. Ethanol and methanol extracts have a faster killing ability at smaller concentrations than N-Hexane extracts [21][5]. *Staphylococcus aureus* is a Gram-positive bacterium, where the cell wall component is polar, while N-Hexane is non-polar. This can also cause N-Hexane extract to be more difficult to diffuse through the cell wall of *S. aureus* bacteria [5][16].

**Table 1.** The MIC Test Extract of Tobacco Flower against *Staphylococcus aureus* and *Candida albicans*

| Microbes | Extract concentration (%) | Extract solvent | | |
|---|---|---|---|---|
| | | Ethanol | Methanol | N-Hexane |
| *S. aureus* | 0 | 0.541 | 1.022 | 1.097 |
| | 2 | 0.538 | 0.548 | 1.018 |
| | 4 | 0.366 | 0.359 | 0.833 |
| | 8 | 0.238 | 0.327 | 0.762 |
| | 16 | 0.205 | 0.225 | 0.533 |
| | 32 | 0.137* | 0.192* | 0.359 |
| | 64 | 0.118* | 0.131* | 0.216* |
| *C. albicans* | 0 | 1.276 | 1.357 | 1.215 |
| | 2 | 1.009 | 1.268 | 1.137 |
| | 4 | 0.728 | 1.102 | 1.092 |
| | 8 | 0.697 | 1.102 | 0.971 |
| | 16 | 0.408 | 1.081 | 0.469 |
| | 32 | 0.210* | 0.264* | 0.406 |
| | 64 | 0.171* | 0.170* | 0.085* |

Note: An asterisk notation (*) indicates clear suspension color

**Table 2.** The MBC Test Extract of Tobacco Flower against *Staphylococcus aureus* and *Candida albicans*

| Microbes | Extract concentration (%) | Extract solvent | | |
|---|---|---|---|---|
| | | Ethanol | Methanol | N-Hexane |
| *S. aureus* | 32 | + | + | + |
| | 64 | - | - | + |
| *C. albicans* | 32 | + | + | + |
| | 64 | - | - | - |

Note: The plus sign (+) indicates bacteria are growing and minus (-) indicates bacteria are not growing.

Ethanol, methanol, and N-Hexane extracts can kill *C. albicans* at a concentration of 64%. The presence of cembranoids content in tobacco is known to function as an antifungal. The inhibitory activity of tobacco flower extract is reported to have higher activity than its leaf extract on *V. mali*. Enzymes that have been isolated from tobacco (chitinase isoforms and β-1,3-glucanase) can damage hyphae and also inhibit hyphal growth of fungi. In another study also mentioned osmotin contained in tobacco can cause lysis of spores, inhibit spore germination, and reduce the ability of spore germination [2].

## CONCLUSION

Ethanol and methanol extracts of tobacco flowers can inhibit the growth and kill *S. aureus* and *C. albicans*. As for the N-Hexane extract of tobacco flowers, it can inhibit the growth and kill *C.albicans* and can only inhibit the growth of *S. aureus*.

## ACKNOWLEDGMENT

Authors thanked to Health Polytechnique of Putra Indonesia Malang for facilitating Pharmacognosy and Microbiology Laboratory for this research.

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