# Antimicrobial activity, Phytochemical Screening of Crude Extracts, and Essential Constituents of *Syzygium Aromaticum, Tymus Vulgaris and Eucalyptus Globulus* on Selected Pathogens

Anubhav Dubey [1*], Mamta Kumari [1]

## Abstract

Background: Plant materials are repositories of bioactive compounds with medicinal value, often utilized in traditional medicines for their therapeutic potential. This study aimed to evaluate the antimicrobial efficacy of extracts from *Syzygium aromaticum*, Thymus vulgaris, and *Eucalyptus globulus* against *Salmonella typhi*, Staphylococcus aureus, *Escherichia coli*, and Candida albicans. Methods: Plant extracts were obtained using soxhlet extraction with ethanol as the solvent. The extracts were qualitatively screened for the presence of alkaloids, saponins, flavonoids, and tannins. The residual concentrations of the plant extracts were determined to be 2.0 mg/mL for *Syzygium aromaticum*, 2.1 mg/mL for Thymus vulgaris, and 1.83 mg/mL for *Eucalyptus globulus*. The antimicrobial activity was assessed, and the minimum inhibitory concentration (MIC) for each extract against the tested microorganisms was determined. Results: All plant extracts contained saponins and flavonoids, while only extracts from *Eucalyptus globulus*, Thymus vulgaris, and *Syzygium aromaticum* contained tannins. Alkaloids were not present in the extracts. *Staphylococcus aureus* was susceptible only to *Syzygium aromaticum* extract. *Salmonella typhi* showed no sensitivity to any of the extracts. *Escherichia coli* was sensitive to all extracts, with the order of efficacy being *Eucalyptus globulus* > Thymus vulgaris > *Syzygium aromaticum*. Candida albicans was sensitive to *Syzygium aromaticum* and Thymus vulgaris extracts. The MIC for *Syzygium aromaticum* was 2.0 mg/mL for S. aureus, E. coli, and C. albicans. Thymus vulgaris exhibited MIC values of 0.021 mg/mL and 2.1 mg/mL for E. coli and C. albicans, respectively. *Eucalyptus globulus* had a MIC of 1.83 mg/mL for E. coli. Conclusion: The plant extracts from *Syzygium aromaticum*, Thymus vulgaris, and *Eucalyptus globulus* demonstrated antimicrobial activity against S. aureus, E. coli, and C. albicans. These findings suggest that these plant materials may be useful as natural remedies for managing infections caused by these susceptible microorganisms. Further research is warranted to explore their potential applications in clinical settings

**Keywords:** *Syzygium aromaticum*, Antimicrobial, *Tymus vulgaris*, Pathogens, *Eucalyptus globulus*.

***Significance*** | This research showed the plant-based treatments' potential as effective alternatives is crucial in combating infectious diseases in an antibiotic resistance condition.

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## Introduction

In addition to synthetic medications, plant-based materials may have additional useful effects in the treatment of infectious disorders (Albertyn et al.,2015; Hosseinzadesh et al., 2015; Jaiswal et al., 2016;).Given the alarming rise in antibiotic resistance, this is

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of the utmost importance. Over the years, plants have developed an enormous variety of secondary metabolites, several of which contain antimicrobial characteristics that can combat various infections caused by bacteria, fungus, and viruses. Researchers have found that a group of naturally occurring substances known as phytochemicals can inhibit various cellular processes in microbes. These compounds include glycosides, tannins, saponins, terpenoids, alkaloids, phenolics, terpenes, anthraquinones, salicylic acid, lupeol, and flavonoids (Okaba et al., 2022; Rojas *et al.,* 2006; Immanuel *et al.,* 2016;). Due to the problem of antimicrobial resistance, there has been a lot of demand for natural substances that possess antibacterial properties. It is thought that the complex and often synergistic interactions between the many bioactive chemicals found in plant extracts make it harder for pathogens to develop resistance (Verpoorte *et al.,* 2017; Zhang *et al.,* 2015;). This is because pathogens would have to beat many defences at once. Using plant materials offers the advantage of employing individual chemicals at lower levels, a crucial aspect for drug safety (Iwu *et al.,* 199;). For a long time, people have used spices like cinnamon, mint, oregano, clove, and mint as food preservatives and medicinal plants due to their antioxidant and antibacterial properties. Numerous recent studies have validated the anticarcinogenic, antifungal, antiviral, and antibacterial characteristics of spice plants. Particularly noteworthy among spices is clove, which has drawn interest owing to its strong antioxidant and antibacterial properties. *Syzygium aromaticum* (*S. aromaticum*) (synonym: *Eugenia cariophylata*), commonly known as clove, is a medium-sized tree (8–12 m) from the *Mirtaceae* family native to the Maluku Islands in east Indonesia. For centuries, clove trade and the search for this valuable spice stimulated the economic development of this Asiatic region (Cortes *et al.,* 2014;).

The genus *Thymus*, a member of the *Lamiaceae* family, contains about 400 species of perennial aromatic, evergreen, or semi-evergreen herbaceous plants with many subspecies, varieties, subvarieties, and forms. In Romania, the Thymus genus contains one species cultivated as an aromatic plant (*Thymus vulgaris*) and another 18 wild species (Boruga *et al.,* 2014). Romanian folk medicine widely uses T. vulgaris (thyme), locally known as "cimbru," for its expectorant, antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative, and diuretic properties.

The *Myrtaceae* family consists of 140 genera and approximately 3800 species distributed in tropical and subtropical regions of the world. Eucalyptus is one of the world's most important and widely planted genera. It is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, and now extensively planted in many other countries. Ramel introduced it to Algeria in 1854. *Eucalyptus* species are well known as medicinal plants because of their biological and pharmacological properties. In the international pharmacopeia, the most important and represented species, however, is *Eucalyptus globulus* (E. globulus), which is the main furnisher of essential oils. These essential oils are highly sought after in the market due to their various applications such as anaesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge, fumigant, haemostats, inhalant, insect repellent, preventive, rubefacient, sedative yet stimulant, vermifuge, and as a folk remedy for abscess, arthritis, asthma, boils, bronchitis, burns, cancer, diabetes, diarrhoea, diphtheria, dysentery, encephalitis, enteritis, erysipelas, fever, flu, inflammation, laryngology es their demand is also high in the soap and cosmetic industries (Bachir *et al.*, 2012;).

The study's goal was to find out how well extracts from *Syzygium aromaticum, Thymus vulgaris, and Eucalyptus globulus killed Salmonella typhi, Staphylococcus aureus, Escherichia coli, and Candida albicans.*

## 2.Materials and Methods

### *2.1 Samples Collection*

The grounds on the Mandhana Campus of Maharana Pratap College of Pharmacy provided the fresh leaves of *Syzygium aromaticum, Tymus vulgaris, and Eucalyptus globulus*. We transported the leaves to the lab in zip-top bags for processing. Fungal pathogen We procured Candida albicans and bacterial *pathogens Salmonella typhi, Staphylococcus aureus, and Escherichia coli* from Aakaar Biotechnology Lucknow, respectively. Following collection, we transferred the isolates to sterilized McCartney bottles and transported them to the lab in an ice box.

### *2.2 Extraction of Phytochemicals*

We rinsed the recovered leaves with distilled water after their delicate separation from the stalk. After drying the leaves of *Syzygium aromaticum, Tymus vulgaris, and Eucalyptus globulus* in a hot air oven for 15 minutes at 40 °C, we crushed them to a fine powder using an SCD-P2030D industrial blender. We pulverized the leaves and added them to 200 millilitres of ethanol in a soxhlet extractor (Figure 1). Next, we let the mixture drain into a clean conical flask. We removed the ethanol by condensing the extract in a rotatory extractor for 10 minutes. To mainstem carefully pipetted 10 millilitres of each extract into a weight watch glass, allowing it to evaporate in a hot water bath until it was completely dry, to maintain a constant weight. placed the dried extracts in a desiccator and sealed them with aluminium foil for a period of seven days. Following the methods outlined by Shah et al. the extracts were tested for the content of alkaloids, glycosides, phenols, saponins, tannins, and flavonoids (Lade *et al.,* 2022).

### *2.3 Determination of Minimum Inhibitory Concentration (MIC)*

Diluting the plant extracts produced 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000 concentrations. After adding ml of each dilution to 10 ml of molten nutritional agar for bacteria and potato dextrose

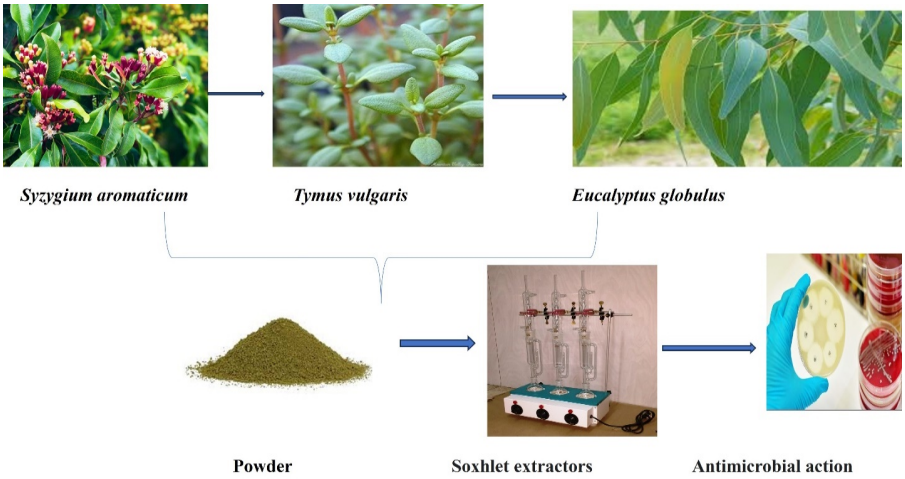


**Figure 1.** Extraction Process of *Syzygium Aromaticum, Tymus Vulgaris* and *Eucalyptus Globulus.*

**Table 1.** Phytochemicals detected in the Plants Extracts

| Plant | Saponins | Alkaloids | Flavonoids | Tannins |
|---|---|---|---|---|
| *Syzygium aromaticum* | + | - | + | + |
| *Tymus vulgaris* | + | + | + | + |
| *Eucalyptus globulus* | + | + | + | + |

*+=Present; −=Absent. The plant extract concentrations after evaporation are shown in Table 2. They are 2.0 mg/ml for Syzygium aromaticum, 2.1 mg/ml for Tymus vulgaris, and 1.83 mg/ml for Eucalyptus globulus.*

**Table 2.** Concentration of plant extract

| Samples | Concentration (mg/ml) |
|---|---|
| *Syzygium aromaticum* | 2.00 |
| *Tymus vulgaris* | 2.10 |
| *Eucalyptus globulus* | 1.83 |

**Table 3.** Sensitivity Test Clinical Isolates to Extracts

| Isolate | *Syzygium aromaticum* | *Tymus vulgaris* | *Eucalyptus globulus* |
|---|---|---|---|
| *Staphylococcus aureus* | ++ | - | - |
| *Salmonella typhi* | - | - | - |
| *Escherichia coli* | + | +++ | ++ |
| *Candidia albicans* | + | + | - |

*= Not sensitive; +=Sensitive*

**Table 4.** Minimum Inhibitory Concentration of Extracts to Clinical Isolates

| Isolate | *Syzygium aromaticum* | *Tymus vulgaris* | *Eucalyptus globulus* |
|---|---|---|---|
| *Staphylococcus aureus* | 2.00 | R | R |
| *Salmonella typhi* | R | R | R |
| *Escherichia coli* | 2.00 | 0.021 | 1.83 |
| *Candidia albicans* | 2.00 | 2.10 | R |

*R – Resistant*

agar for fungus, I let it set. I streaked each test isolate across the plate surface and incubated it for 24 hours at 37 °C. As the MBC, the extract concentration that stopped microbial growth was selected (Biswas *et al*., 2013;).

***2.4 Susceptibility Test***

We impregnated discs of sterile paper with the resulting solutions of 1:2, 1:3, and 1:4. Twenty minutes was enough time for the discs to soak up the extract. We prepared agar plates with lawns of test isolates using sterile swab sticks. We placed the prepared discs on the plates, spacing them 2 cm apart, and then incubated the plates for 24 hours at 37 °C. We used the zone of inhibition to determine the sensitivity of the test organisms (Ocean *et al.,* 2015; Gurung *et al.,* 2020; White *et al.,* 2002;).

## 3. Results

***3.1 Phytochemicals***

Table 1 and table 2 display the phytochemical profile of the plant extracts. Saponins, alkaloids, flavonoids, and tannins are present in *Syzygium aromaticum*; they are also present in *Tymus vulgaris and Eucalyptus globulus*, as well as alkaloids and flavonoids.

3.2 ***Sensitivity of the test organisms***

Table 3 displays the test organisms' sensitivity patterns to the ethanol extracts of *Eucalyptus globulus, Tymus vulgaris, and Syzygium aromaticum. Syzygium aromaticum* was the only pathogen that could infect *Staphylococcus aureus*. Not every extract proved effective against *Salmonella typhi*. All extracts from the order *Tymus vulgaris* > *Eucalyptus globulus* > *Syzygium aromaticum* were susceptible to *Escherichia coli*. Extracts from *Tymus vulgaris and Syzygium aromaticum* were susceptible to *Candidia albicans*.

***3.3 Minimum Inhibitory Concentration of Extracts***

Table 4 displays the MIC of extracts from *Tymus vulgaris, Eucalyptus globulus, and Syzygium aromaticum* against test organisms. For S. aureus, E. coli, and C. albicans, the MIC of *Syzygium aromaticum* was 2.0 mg/ml, respectively. For E. coli and Candida albicans, the MIC of *Tymus vulgaris* was 0.021 mg/mL and 2.1 mg/mL, respectively. For *Eucalyptus globulus, E. coli* has a MIC of 1.83 mg/mL.

## 4. Discussion

This study evaluated the antibacterial qualities of three medicinal plants: *Eucalyptus globulus, Tymus vulgaris, and Syzygium aromaticum*. Because medicinal plants contain chemicals commonly found on leaves, stems, fruits, and seeds, they provide therapeutic benefits. We subjected plant extracts to phytochemical screening, verifying the presence of flavonoids, tannins, alkaloids, and saponins. Depend Plants can cure disorders either alone or in combination, depending on their phytochemical composition. Saponins, alkaloids, flavonoids, and tannins are present in *Syzygium aromaticum*; they are also present in *Tymus vulgaris and Eucalyptus globulus*, as well as alkaloids and flavonoids. This is in line with studies on these plants' phytochemical screening.

The investigation's findings showed that the leaves of *Syzygium aromaticum, Tymus vulgaris*, or *Eucalyptus globulus* did not inhibit *Salmonella typhi*, but they did inhibit several test organisms, including *Staphylococcus aureus, Escherichia coli, and Candidia albicans*. These leaves contain flavonoids, alkaloids, tannins, and saponins, which have demonstrated their ability to inhibit pathogenic bacteria (Okaba *et al.,* 2022; Rojas *et al.,* 2006; Immanuel *et al.,* 2016;).

For S. aureus, E. coli, and C. albicans, the MIC of *Syzygium aromaticum* was 2.0 mg/ml, respectively. For *E. coli and Candida albicans*, the MIC of *Tymus vulgaris* was 0.021 mg/mL and 2.1 mg/mL, respectively. The MIC of E. coli for *Eucalyptus globulus* was 1.83 mg/mL. The MICs of the extract for *S. aureus, E. coli, and C. albicans* are significantly lower than the 25.0 mg/ml reported by (Okaba *et al.,*2022).

Important human infections, including *Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Candida albicans*, have demonstrated rising antibiotic resistance, creating a major problem for clinical management and public health. Research has shown that commonly used antibiotics such as ampicillin, beta-lactams, carbapenems, chloramphenicol, fluoroquinolones, trimethoprim-sulfamethoxazole, fluconazole, clotrimazole, and ketoconazole exhibit resistance (Ocean *et al.,* 2015; Gurung *et al.,* 2020; White *et al.,* 2002;).These antibiotics were previously effective in treating infectious diseases brought on by *Salmonella typhi, Staphylococcus aureus, Candida albicans, and Escherichia coli*. It is hoped that these viruses are susceptible to plant extracts.

## 5. Conclusion

Researchers discovered alkaloids, tannins, flavonoids, and saponins in the ethanol extracts of *Eucalyptus globulus*, *Tymus vulgaris, and Syzygium aromaticum*. The extracts demonstrated antibacterial efficacy directed against *Candidia albicans, Escherichia coli, and Staphylococcus aureus*. We can utilize extracts from *Tymus vulgaris, Eucalyptus globulus, and Syzygium aromaticum* to create new antimicrobial compounds that will fight infectious disorders caused by vulnerable microbes.

### Author contributions

A.D. was responsible for drafting the original manuscript. M.K. contributed to the analysis, data interpretation, and revisions. Both authors reviewed and approved the final version of the manuscript.

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## Competing financial interests

The authors have no conflict of interest.

## References


Albertyn, R., Berg, A., Numanoglu, A., & Rode, H. (2015). Traditional burn care in sub-Saharan Africa: a long history with wide acceptance. Burns : journal of the International Society for Burn Injuries, 41(2), 203–211. https://doi.org/10.1016/j.burns.2014.06.005

Alqahtani, A. S., & Al-Johani, N. S. (2020). Antimicrobial activity of ginger (Zingiber officinale) against multidrug-resistant clinical isolates. Saudi journal of biological sciences, 27(8), 2083–2088. https://doi.org/10.1016/j.sjbs.2020.03.008

Bachir, R. G., & Benali, M. (2012). Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and Staphylococcus aureus. Asian Pacific journal of tropical biomedicine, 2(9), 739–742. https://doi.org/10.1016/S2221-1691(12)60220-2

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., & Yadav, A. (2013). Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria. International journal of microbiology, 2013, 746165. https://doi.org/10.1155/2013/746165

Borugă, O., Jianu, C., Mişcă, C., Goleț, I., Gruia, A. T., & Horhat, F. G. (2014). Thymus vulgaris essential oil: chemical composition and antimicrobial activity. Journal of medicine and life, 7 Spec No. 3(Spec Iss 3), 56–60.

Cortés-Rojas, D. F., de Souza, C. R., & Oliveira, W. P. (2014). Clove (*Syzygium aromaticum*): a precious spice. Asian Pacific journal of tropical biomedicine, 4(2), 90–96. https://doi.org/10.1016/S2221-1691(14)60215-X

Costa-de-Oliveira, S., & Rodrigues, A. G. (2020). Candida albicans Antifungal Resistance and Tolerance in Bloodstream Infections: The Triad Yeast-Host-Antifungal. Microorganisms, 8(2), 154. https://doi.org/10.3390/microorganisms8020154

Gurung, R. R., Maharjan, P., & Chhetri, G. G. (2020). Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from pediatric patients. Future science OA, 6(4), FSO464. https://doi.org/10.2144/fsoa-2019-0122

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A. and Armand, R. (2015) The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. International Journal of Clinical Medicine, 6, 635-642. http://dx.doi.org/10.4236/ijcm.2015.69084

Immanuel OM., Abu GO., Stanley HO. (2016). Inhibition of biogenic sulphide production and biocorrosion of carbon steel by sulphate reducing bacteria using Ocimum gratissimum essential oil. Journal of Biology & Biotechnology.10(2): 1-12.

Iwu, M.W., Duncan, A.R. and Okunji, C.O. (1999) New Antimicrobials of Plant Origin. In: Janick, J., Ed., Perspectives on New Crops and New Uses, ASHS Press, Alexandria, 457-462.

Jaiswal, Y., Liang, Z., & Zhao, Z. (2016). Botanical drugs in Ayurveda and Traditional Chinese Medicine. Journal of ethnopharmacology, 194, 245–259. https://doi.org/10.1016/j.jep.2016.06.052

Kamboj, V. P. (2000). Herbal medicine. Current Science, 78(1), 35-39.

Kannan, S., & Saravanan, R. (2020). In vitro antibacterial and antifungal activities of ethyl acetate extract of Aloe vera leaf gel against human pathogens. Journal of King Saud University-Science, 32(1), 713–717. https://doi.org/10.1016/j.jksus.2018.03.017

Lade SN., Burle SS., Kosalge S.B, Bansode MN. (2022).Antimicrobial and Antioxidant activity of Hibiscus Sabdariffa. linn (Roselle) International Journal of Pharmacy Research & Technology. 12(1): doi: 10.31838/ijprt/12.01.04

Manosroi, J., Jantrawut, P., & Manosroi, A. (2008). Antimicrobial activity of essential oils from Thai medicinal plants against food-borne pathogens and spoilage bacteria. Food Science and Technology International, 14(5), 489–496. https://doi.org/10.1177/1082013208097235

Naghibi, F., Mosaddegh, M., Motamed, S. M., & Ghorbani, A. (2005). Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iranian Journal of Pharmaceutical Research, 2(2), 63-79.

Ocean, H. O., Eze, E. A., Eze, C. N., & Enebe, M. C. (2015). Drug resistance pattern of Salmonella and Shigella species isolated from selected hospitals in Anyigba, Kogi State, Nigeria. African Journal of Microbiology Research, 9(37), 2023-2036.

Okaba AE., Immanuel OM., Stow, KM., Oku IY. (2022).Evaluation of the antimicrobial activities of selected plant extracts and honey against clinical isolates. African Scientist. 23:(3) 30: 175-179.

Rojas, J. J., Ochoa, V. J., Ocampo, S. A., & Muñoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. BMC complementary and alternative medicine, 6, 2. https://doi.org/10.1186/1472-6882-6-2

Ugboko HU., Nwinyi OC., Oranusi SU., Fatoki TH., Omonhinmin CA. (2020). Antimicrobial importance of medicinal plants in Nigeria. Sci. World J. 1 (1): 1-10, 10.1155/2020/7059323

Verpoorte D. R. (2017). New times for traditional medicine research. Journal of ethnopharmacology, 197, 1. https://doi.org/10.1016/j.jep.2017.01.018

Zhang, J., Onakpoya, I. J., Posadzki, P., & Eddouks, M. (2015). The safety of herbal medicine: from prejudice to evidence. Evidence-based complementary and alternative medicine : eCAM, 2015, 316706. https://doi.org/10.1155/2015/316706