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# Determination of antibacterial activity of *Syzygium aromaticum* on *Escherichia coli* and *Staphylococcus aureus*.

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## Abstract

Plant extracts are widely utilized for therapeutic purposes since they are inexpensive, effective, safe and have no or very less negative effects. Spices are components of several plants that add food preparations a distinct scent and flavor. The goal of this study was to discover if *Syzygium aromaticum* bud and leave extracts have antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Clove bud and clove leave extracts were made using distilled water and 100 % ethanol.Clove leave extract using ethanol extract showed maximum antimicrobial activity. The antimicrobial properties of preservation based on essential oils was also investigated by storing them for 7 days. According to disk diffusion test essential oils has exhibited antibacterial activity against *S. aureus* and *E. coli*, both alone and in combination with medicines. Storage influenced the two spices as well, with an overall decrease in activity. The ethanol extract of clove leaves had the highest activity index 38.3 against *E. coli*. To measure the Minimum Inhibitory Concentration (MIC) of essential oils broth dilution was used. The MIC was observed in $10^{-2}$ µl/ml concentration in both *S.aureus* and *E.coli*. For the minimum bactericidal concentration (MBC) colonies didn't appear,indicating bactericidal activity of the sample.

**Keywords:** *Syzygium aromaticum*, Antibacterial activity, MIC, MBC

## 1. Introduction

In the twenty-first century, multidrug-resistant (MDR)microorganisms pose a huge threat to human health.[1] The WHO published a report in 2017 mentioning the most dangerous MDR bacteria. Antibiotics have resulted in a massive surge in antibiotic resistance among several bacterial infections. Antimicrobial resistance (AMR) develops in microorganisms because of an infection that has a high incidence and might last for a great many years of antibiotic subjection. So, alternative approaches are needed to cure the diseases caused by these resistant organisms. New antibiotics are mostly discovered using natural compounds generated from natural sources. Researchers figured that plant extracts as an alternative to the common antibiotics.

Herbal remedies are extracted and processed for used in scientific research. When preparing medicinal plants for research purposes, must focus the timely and proper gathering of the plant, expert authentication, suitable drying, and grinding.[2] We must follow bioactive separation, stratification, and segregation of molecule, if applicable. It mainly comprises determination of the bioactive compounds. Plants have recently gained significant popularity as a source of medication due to their natural origin, availability in surrounding communities, cheaper cost, ease of administration, and maybe less bothersome nature, and acting as a different treatment strategy

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for drug resistance. The extraction method is selected considering the content of the leaf tissue, the fluid employed, the acid levels of the fluid, its warmth, or the liquid to material relation.

Specially, spice medical plants are widely used for flavoring food. Most Asian countries have a long history of traditional medical systems and a diverse spectrum of spices used in therapies. Many spices have antibacterial capabilities due to presence of a variety of chemicals and metabolites present in plant body. Since ancient times, spices have long been used as condiments and additives in ceremonies. Due to their antibacterial characteristics, many spices, such as clove, oregano, thyme, cinnamon, and cumin, are being used to treat several diseases.[3] Spices are typically used to give food scent, flavor, or piquancy, as well as to season it.

Essential oils (EOs) are very promising compounds for producing new antibacterial drugs. Previous research studies have reported a strong antibacterial effect for some EOs. EOs are complex combinations of aromatic plants' secondary metabolites. EOs are known for their bactericidal, antiviral, and fungicidal capabilities, as well as medicinal and aromatic characteristics. EOs has antibacterial, analgesic, sedative, and anti-inflammatory properties. Perfumes, make-up, health, dentistry, and agricultural products all include Eos.[4] EOs can be extracted from a variety of plant organs, including flowers, leaves, fruits, seeds, barks, and roots. The composition and extracted yield of EOs are said to be dependent on the species, soil composition, extraction method, and extraction condition, according to prior study publications. Cold pressing, hydro-distillation, crude extraction, maceration and steam distillation are few methods used to extract EOs. *Syzygium aromaticum* buds and leaves essential oil were obtained using several solvent extraction procedures and it was determined which solvent was the most suitable for each sample.

Menstruum is a solution that is used to separate medicinal herbs.[5] When we are choosing the solvent; crop type, the part of the plant, the nature of the phytochemical compounds, and the solvent supply must all be considered. In addition to solvent selectivity, safety, reactivity, recovery, and viscosity.

Clove belongs to Myrtaceae family. It is commonly utilized to combat oral bacteria's infections.[6] Due to its powerful antibacterial qualities against different food-related disorders, it is utilized in the as a natural additive to improve lifespan. The antimicrobial properties of clove hydro distillation and several solvent extraction methods against *Staphylococcus aureus* and *Escherichia coli* were investigated in-vitro and in-vivo. Clove oil (CO) has biological properties. and used as an antiseptic to treat oral infections. Mold, yeast, and microbes have all been found to be suppressed by CO. In tryptone soya broth and cheese, it was efficient against *L. monocytogenes* and *S. enteritidis*. Both eugenol and the phenolic components of CO keep interaction between cell membrane as well.

The liquid medium evaporates during extraction, releasing a concentrated form of an active component. The isolates or essential oils obtained from seasonings and cuisines are determined by the type of extraction or procedure used. These extracts and oils are often evaluated for antibacterial activity using the agar disk diffusion method. The minimum inhibitory concentration (MIC) is used to determine an organism's sensitivity.[7] When compared to untreated, the MIC is defined as the antibacterial component with the lowest concentration after a particular duration of incubation at a specific temperature, usually $37^0$C. According to a method demonstrated by Valgas and colleagues' various pathogens have different MICs for different extracts dilutions.

To determine which solvent was the most successful for the bacteria, essential oil from *S.*

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*aromaticum* was extracted using different solvent extraction methods. Antibiotic sensitivity test on *E. coli* and *S. aureus* to determine susceptibility to the gentamycin.

This study looked at the influence percentage mycelial growth suppression against gram positive bacteria of herbal extracts spherical shape *S.aureus* and gram negative rod form *E.coli*.[8]

Over 80% of the global utilizes herbal treatments. Indeed, several natural products have been used as lead compounds in the development of extremely physiologically relevant chemicals, pharmacological compounds that are semi-synthetic and have a higher potency for therapeutic application, efficiency and efficacy are important.[9] The presence of tannins, saponins, and essential oils in these spices may contribute to their antibacterial properties, oils, flavonoids, and phenolic compounds. Even crude extracts of these spices are effective against multidrug resistant bacteria that have become resistant to current antibiotics.

## 2. **Methodology**

*2.1 Sample preparation.* Clove bud samples randomly collected from an ayurvedic shop were cleaned thoroughly to remove contaminants. After that, using a mortar and pestle, a good quality clove was ground.[10] 50g of each sample was weighed and processed in a spice grinder until a uniform powder was obtained. Soxhlet apparatus was used to prepare each sample within 24 hours of the essential oil.

*2.2 Extraction of essential oils from clove powder using Hydro distillation method.* Essential oil extraction was carried out in accordance with the procedure outlined by Muhamed *et al*. A total of 40g of material has been generated.[11] The boiling flask was placed on the hotplate and was heated to $1000^0$C.Fluid remained visible after adding 150ml of distilled water. After that samples were centrifuged at 2500 rpm for 10 minutes. After that, the oil was placed in a mini centrifuge tube. The mass of the generated oil was measured to determine the yield. It was then kept at $40^0$C until needed.[12]

*2.3 Extraction of essential oils.* 40g clove leaves were collected from a local plant nursery and was grounded using mortar and pestle. Then 100ml of 95% ethanol was mixed in 2:5 ratio (ground leaves:ethanol). The oil was then poured into a 1.5ml microcentrifuge tubes. To estimate the productivity, the bulk of the created oil was weighed using an analytical balance.[13]

*2.4 Preparation of Bacterial dilutions (MacFarland standard).* 1% $H_2SO_4$ was prepared. Then 1% $BaCl_2$ was also prepared. Then, to make the 10ml Mc- Farland standard, $H_2SO_4$ and $BaCl_2$ was mixed and stirred thoroughly.[14]Then using spectrophotometer absorbance was checked at 625nm. McFarland standard absorbance range could be 0.08 and 0.10.

*2.5 Disc diffusion method for fresh extracted essential oil.* Muller-Hinton agar plates were divided into three quadrants. Each quadrant was used for positive control (P), negative control (N) and sample ($S_{01}$), *E.coli* and *S.aureus* were streaked agar plates. Then filter paper discs were placed in negative and $S_{01}$ quadrant[15]. Sterile, distilled water infused filter paper disks were used as negative controls. Gentamycin was used as positive control. After that, the inoculated petri dishes were incubated at $37^0$C for 24 hours. Finally, the zone of inhibition was measured.

*2.5.1. MIC broth dilution method for extracted clove leave oil.* Six test tubes were prepared by adding 9ml of nutrient broth following the method described by Fauzya *et al*, 2ml of concentrated essential oil was added to the first tube and was labeled as the sample.[16] From the sample test tube 1ml essential oil was serially transferred to the rest of the tubes. Then, 2µL

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*S.aureus* was added each test tube. For the control 9ml nutrient broth and *S.aureus* was added. Same procedure was followed for *E.coli*.[17]

*2.5.2 Minimum Bactericidal Concentration (MBC) method for extracted clove leave oil*. Tryptone Soy Agar was spread inoculated using 10µL from last three each dilution. Same procedure was followed for *S.aureus* dilutions.[18] Then the plates were incubated at $37^0$C for 24 hours.

## 3. Results and Discussion

3.1 Soxhlet Extraction of *Syzygium aromaticum*. The essential oil was extracted from the clove bud using the hydro distillation. The yield was estimated by weighing the essential oil mass. (Table 1)

**Table 1.** Volume and yields (w/w %) of essential oils obtained by hydro distillation. [19]

| Sample | Volume of oil (mL) | Mass of oil (g) | Mass of sample used (g) | Yield (w/w %) |
|---|---|---|---|---|
| *Syzygium aromaticum* (Clove bud) | 2.50ml | 2.708g | 40g | 2.708/40×100 =6.77% |

*3.2 Crude extraction of Syzygium aromaticum.* Powdered plant materials were placed in a container. Samples were filled with menstruum. Then the falcon tubes were allowed to mix on the roller mixture for 24 hours to ensure complete extraction. After the completion of the extraction, the extract was decanted. This method is simple to implement and works well with thermo labile leaf tissue.[20]

The yield was determined by monitoring the weight of essential oil extracted from clove leaves using the crude extraction method (Table 1).[21]

**Table 2.** Volume and yields (w/w %) of essential oils obtained by hydro distillation.

| Sample | Volume of oil (mL) | Mass of oil (g) | Mass of sample used (g) | Yield (w/w %) |
|---|---|---|---|---|
| *Syzygium aromaticum* (Clove leave) | 2.50ml | 1.5g | 40g | 1.5/40×100 =3.75% |

P (+) – Positive control

N(-) – Negative control

$S_y$-Essential oil +Positive control

$S_{01}$– Sample essential oil

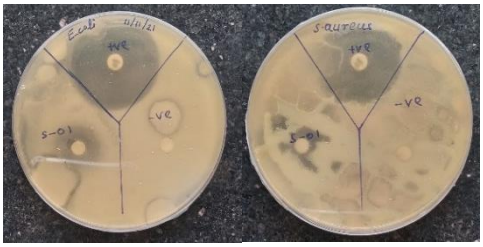
$E_o$- Essential oil

*3.3 Antibacterial Susceptibility Test*

*3.3.1. Antibacterial activity of Syzygium aromaticum (Clove bud) oil*

P (+) – Positive control N(-) – Negative control

$S_{01}$– Sample essential oil




A B

**Figure 1.** Zones of inhibition fresh clove bud oil trial o1 *E.coli* (A) and 1 week old clove bud oil (B) against *S aureus*

A B

**Figure 2.** Zones of inhibition 1 week old clove bud oil trial-02 *E. coli* (A) and 1 week old clove bud oil (B) against *S aureus*, with Gentamycin as the positive control.

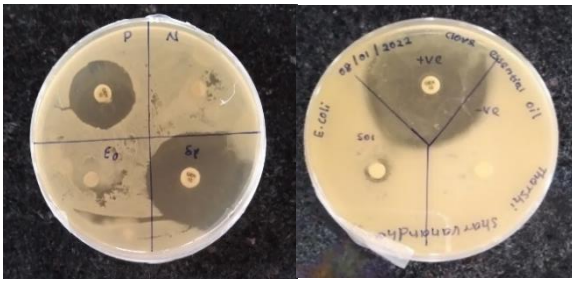
P (+) – Positive control

N(-) – Negative control

$S_y$-Essential oil +Positive control

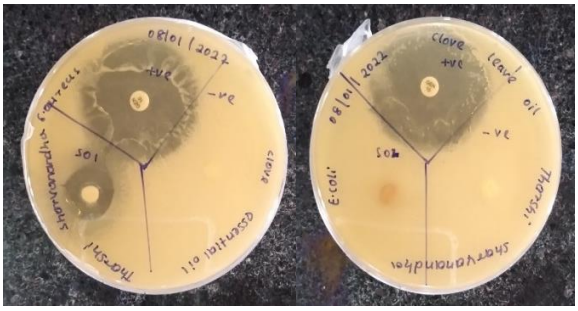
$S_{01}$– Sample essential oil

$E_o$- Essential oil



A B

**Figure 3.** Zones of inhibition fresh clove leave oil *E. coli* (A) and 1 week old clove bud oil (B) against *S aureus*, with Gentamycin as the positive control.



A B

**Figure 4.** Zones of inhibition fresh clove leave oil *E. coli* (A) and 1 week old clove leave oil (B) against *S aureus*, with Gentamycin as the positive control.

The zones of inhibition for essential oil extracted from *Syzygium aromaticum* against *S aureus* and *E coli* was observed.

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**Table 3.** Zones obtained for *S aureus* antibacterial activity of Clove bud oil.

| | Clove bud oil (Trial 01 mm) | Clove bud oil (Trial 02 mm) | Clove bud oil (Trial 03 mm) | Mean (mm) |
|---|---|---|---|---|
| Negative control | 0.00 | 0.00 | 0.00 | 0.00 |
| Gentamycin | 32 | 35 | 35 | 34 |
| Clove bud oil | 14 | 19 | 18 | 17 |

**Table 4.** Zones obtained for *E. coli* antibacterial activity of Clove bud oil.

| | Clove bud oil (Trial 01 mm) | Clove bud oil (Trial 02 mm) | Clove bud oil (Trial 03 mm) | Mean (mm) |
|---|---|---|---|---|
| Negative control | 0.00 | 0.00 | 0.00 | 0.00 |
| Gentamycin | 32 | 35 | 35 | 34 |
| Clove bud oil | 15 | 17 | 16 | 16 |

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**Table 5.** Zones obtained for clove leaf oil antibacterial activity against *S aureus.*

| | Clove leaf oil (Trial 01 mm) | Clove leaf (Trial 02 mm) | Clove leaf oil (Trial 03 mm) | Mean (mm) |
|---|---|---|---|---|
| Negative control | 0.00 | 0.00 | 0.00 | 0.00 |
| Gentamycin | 35 | 35 | 35 | 35 |
| Clove leaf oil | 33 | 23 | 25 | 27 |

**Table 6.** Zones obtained for clove leaf oil antibacterial activity against *E.coli.*

| | Clove leaf oil (Trial 01 mm) | Clove leaf (Trial 02 mm) | Clove leaf oil (Trial 03 mm) | Mean (mm) |
|---|---|---|---|---|
| Negative control | 0.00 | 0.00 | 0.00 | 0.00 |
| Gentamycin | 35 | 35 | 35 | 35 |
| Clove leaf oil | 43 | 44 | 28 | 38.3 |

**Table 7.** Comparison of zone of inhibition in the clove bud oil and clove leaf oil samples.

| Samples | Average zone of inhibition (mm) | |
|---|---|---|
| | *S.aureus* total mean value | *E.coli* total mean value |
| Clove bud oil | 17 | 16 |
| Clove leaf oil | 27 | 38.3 |

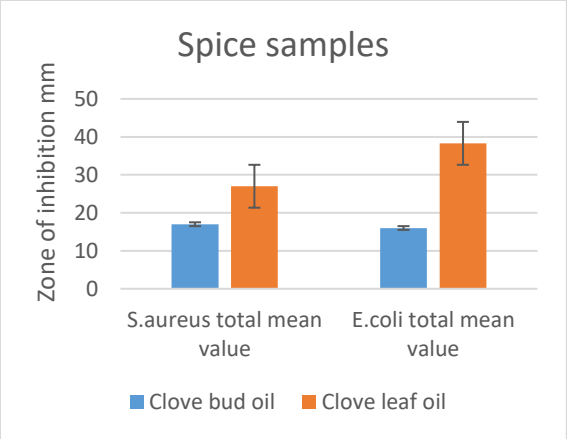


**Figure 5.** Graph representation of the zone of inhibition in the clove bud oil and Clove leaves oil sample.

*3.4 Minimum Inhibitory Concentration*

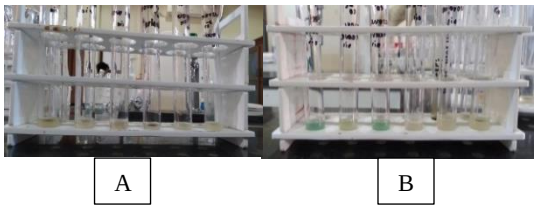


**Figure 6.** Minimum Inhibitory concentration of *S.aureus* dilutions (A) Minimum inhibitory concentration of *E.coli* dilutions (B).

Control-Nutrient broth and bacteria.

The MIC was observed in $10^{-2}$ µl/ml concentration in both *S.aureus* and *E.coli* dilutions.

*3.5 Minimum Bactericidal Concentration.* The clones were not observed in both samples, hence they could be bactericidal


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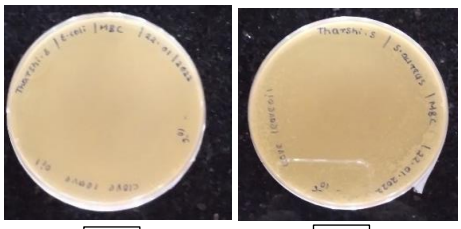
**Figure 7.** MBC of fresh clove leave oil *S.aureus* (A) and 1 week old clove leave oil (B) against *E.coli*



A B

**Figure 8.** MBC of fresh clove leave oil *E. coli* (A) and 1 week old clove leave oil (B) against *S aureus.*

## 4. Discussion

Plant extracts, for example, open a new horizon for the discovery of novel medicinal molecules. Traditional medicine and medicinal herbs are widely used in most before scientific.[22] It has been commonly noticed that countries serve as a normative underpinning for maintaining good health.[23]

As a result, the antibacterial activity of clove bud and leaves was investigated against *E. coli* and *S.aureus* in this study.[24] The practical approach of utilizing disk plate assays to evaluate relative antibacterial activity based on the size of the inhibitory zone is insufficient to determine the antibacterial nature of these spices.[25] A thorough scientific investigation of traditional spices is necessary to ensure that they are effective against a wide range of bacterial species, as this would provide scientific support for their use. The solubility and velocity of diffusion in agar determine the clear zone medium and the process of volatilization.[26]

In the methodology, samples were crushed to increase the surface area. Ethanol was used in the extraction since ethanol is an effective solvent when compared with other solvents, hence can end up with high yield of essential oil.[27] Hydro distillation with powdered clove buds yielded a total amount of 2.50ml, with a return of 6.77%. Clove bud has the highest yield.[28]

Numerous research has been conducted to date in order to assess the antibacterial potential of various essential oils against a variety of microbes. Antibacterial phytochemicals included in essential oils may be useful against bacterial infections.[29] Biologists are reviving the usefulness of spices and condiments through in-vitro and in-vivo research that antibacterial activity, notably in Savita *et al*, 2017 antimicrobial activity of essential oils are used as alternative antimicrobial remedy. (*S.aureus*)[30] and (*E.coli*), can cause super skin infections and life-threatening diseases like endocarditis and sepsis, cholangitis, and urinary tract infection (UTI).[31]Antibacterial susceptibility test (ABST) is extensively used in clinical settings to assess antimicrobial resistance characteristics of test organisms, guide antibiotic treatment options, to identify antibiotic resistance.[32] The Kirby-Bauer test, also referred as disk diffusion method is a classical microbiology technique that is quite well used. The disk diffusion method for determining antimicrobial resistance is likely the most widely utilized, around the world due to its ease, efficiency, and low cost. It was carried out with the use of gentamycin as positive controls.[33]

Tables 3 and 4 of the ABST results for CO showed zones of radius of 17 and 16mm, respectively, which shows similar values in prior research studies carried out by.[34] Fresh clove oil inhibited *S.aureus* and *E.coli* in a synergistic manner. When compared to clove alone, when combination with Gentamycin 34mm.[35]

The presence of tannins, saponins, and essential oils in these spices may contribute to their antibacterial properties.[36] Oils, flavonoids, and phenolic compounds. Even crude extracts of these spices are effective against MDR bacteria that are resistant to current antibiotics. But the antibiotic effect observed in this work was limited. This study cannot predict the effect of these spices on these species in vivo.[37] The

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practical approach of utilizing disk plate assays to determine relative antibacterial activity based on the size of the inhibition zone is insufficient to determine the antibacterial nature of these spices.[38] The clear zone is determined by the diffusion velocity and solubility in agar. A comprehensive scientific study of traditional spices is necessary to understand the medium and the volatilization process that results from it.[39] Must guarantee that they are effective against a wide range of bacterial species, and this would give scientific support for their use their effectiveness.[40]

ABST results observed for clove leave oil showed Table 5 and Table 6with zone of radius of 5mm,5mm respectively, which showed similarities with CLSI standard and previous studies. Based on this finding, it may be concluded that leave oil contains more eugenol anti-bacterial than bud oil.[41]

The MIC is the minimum antibacterial quantity that will suppress apparent bacterial growth during an overnight incubation period. MICs are mostly employed in labs to confirm resistance, but they are also frequently used in research in order to assess the in-silico activities of novel antimicrobials, with the results of these studies used to determine MIC breakpoints. The MIC experiments were carried out by essential oils were used in various concentrations, such as $10^{-1}$,$10^{-2}$,$10^{-3}$,$10^{-4}$,$10^{-5}$µl/ml.[42] In MIC of *S.aureus* dilutions in $10^{-2}$ µl/ml the turbidity was reached and *E.coli* dilution $10^{-2}$ µl/ml the turbidity was reached. It showed certain similarities to earlier research.[43]

The lowest antimicrobial concentration that will prevent an organism from developing following antibiotic-free medium are used for cultures known as the MBC. In tryptone soy agar plates the colonies were not visible, possibly due to bactericidal effect.[44]

## 5. Conclusion

Research focus on the essential oils of clove bud and clove leaves were extracted by hydro distillation and crude extraction, and the lower yields of essential oils obtained were consistent with previous research. Fresh and 7-day-old extracted essential oils were tested for antibacterial activity, and due to storage lowered activity was observed in both clove bud and clove leave oil extracts.[45] Fresh and 7-day preserved essential oils of clove bud and clove leaves have varying synergistic and antagonistic effects when combined with gentamycin against both bacterial species.[46] The action of the antimicrobial ethanolic extract of clove leaves was highest against both gram negative and gram-positive bacteria. Although all the spices studied have antibacterial properties, the degree of antibacterial activity differs by species. The ethanol extract of clove leaves had the highest activity index 38.3 against *E. coli*. The Design of experiments (DOE) study offered a fascinating and potentially useful low-cost method for combining spice and herb extracts in the ideal ratios to get the most biological impact. Thus, the DOE opened a creative space for the next generation of research and development of the best antimicrobial compounds for the food and pharmaceutical industries.[47]

## Acknowledgements

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## References


1. D. Zhang, R.Y. Gan, A.K. Farha, G. Kim Yang, X.M. Shi, C.L. Luo, Q.X. Xu, H.B Li and H. Corke, *Microorganisms*, 2019;**7**(6); p.157.
2. H.J. Sohilait, H. Kainama and M. Nindatu. *International Journal of Organic Chemistry*, 2018;**8**(2);229-39.
3. P. Falcone, B. Speranza, M.A. Del Nobile, M.R. Corbo and M. Sinigaglia. *Journal of food protection*, 2005;**68**(8);1664-70.
4. A. Banik, M. Abony, T. Zerin and S. Datta. *IOSR Journal of Pharmacy and Biological Sciences*, 2018;**13**;68-73.
5. H. Mith, R. Dure, V. Delcenserie, A. Zhiri, G. Daube and A. Clinquart. *Food science & nutrition.* 2014;**2**(4);403-16.
6. J.M. Andrews. *Journal of antimicrobial Chemotherapy*, 2001;**48**;5-16.
7. A. Al-Mariri and M. Safi. *Iranian journal of medical sciences*, 2014;**39**(1);36.
8. J. Redfern, M. Kinninmonth, D. Burdass and J. Verran. *Journal of microbiology & biology education*, 2014;**15**(1);45-6.
9. J.Y. Lim, J.W. Yoon and C.J Hovde. *Journal of microbiology and biotechnology*, 2010;**20**(1);5.
10. K. Syal, M. Mo, H. Yu, R. Iriya, W. Jing, S. Guodong, S. Wang, T.E. Grys, S.E. Haydel and N. Tao. *Theranostics*. 2017;**7**(7);1795.
11. L.G Hagh, A. Arefian, A. Farajzade, S. Dibazar and N. Samiea. *Dental Research Journal*, 2019;**16**(3);153.
12. L. Jirovetz, G. Buchbauer, I. Stoilova, A. Stoyanova, A. Krastanov and E. Schmidt. *Journal of agricultural and food chemistry*. 2006;**54**(17);6303-7.
13. B. Shan, Y.Z. Cai, J.D. Brooks and H. Corke. *International Journal of food microbiology*, 2007;**117**(1);112-9.
14. S.A. Kadhem Salman, S.O. Hasson, N.R Abady and H.K Judi, *Annals of the Romanian Society for Cell Biology*, 2021;7476-86.
15. A.A. Khalil, U. Rahman, M.R. Khan, A. Sahar, T. Mehmood and M. Khan, *RSC Advances*, 2017;**7**(52);32669-81.
16. L. Nuñez and D.M. Aquino. *Brazilian Journal of Microbiology*, 2012;**43**;1255-60.
17. M. Daker, V.Y. Lin, G.A. Akowuah, M.F. Yam and M. Ahmad. *Experimental and therapeutic medicine*, 2013;**5**(6);1701-9.
18. M. Khatun, M.A. Nur, S. Biswas, M. Khan, and M.Z. Amin. *Journal of Agriculture and Food Research*, 2021;**6**:100201.
19. S.N. Sah, H. Khanal and D.R. Acharya. *Journal of Microbiology*, 2020;**7**;8-18.
20. A.F. Fauzya, R.I Astuti and N.R Mubarik. *International Journal of Microbiology*, 2019;2019.
21. S.A. Mamza, Y.A. Geidam, G.D. Mshelia, G.O. Egwu and I. Gulani. *Direct Res. J*, 2016;**1**(1);1-8.
22. S. Revati, C. Bipin, P.B. Chitra and B. Minakshi. *Arch Med Sci*, 2015;**11**(4);863-8.
23. Y.O. Adesiji, O.A. Shittu and A.S. Oluremi. *Nigerian Journal of Pure & Applied Science*, 2015;**28**;2610–2616.
24. M.L. De Castro and F. Priego-Capote. *Journalof chromatography*, 2010;**1217**(16):2383-9.
25. J.L. Romero, M.J. Grande, R. Burgos,Pérez-Pulido and A. Gálvez. *Frontiers in microbiology*, 2017;**31**(8);1650.
26. D. Maharjan. *The study of Antibacterial Activity of Common Spices* (Doctoral dissertation, Department of Microbiology).
27. N. Błaszczyk, A. Rosiak and J. Kałużna-Czaplińska. *Forests*, 2021;**12**(5)648.
28. J.N. Haro-González, G.A. Castillo-Herrera, M. Martínez-Velázquez and H. Espinosa-Andrews, *Molecules*, 2021;**26**(21);6387.
29. N.N. Azwanida. *Med Aromat Plants*, 2015;**4**(196);2167-0412.
30. N.N. Kasim, S.N. Ismail, N.D Masdar, F.A Hamid and W.I Nawawi. *International Journal of Scientific and Research Publications*, 2014;**4**(7);2250-3153.
31. S. Nagalakshmi, P. Saranraj and P. Sivasakthivelan. *Journal of Drug Delivery and Therapeutics*, 2019;**9**(3);33-5.
32. P. Yap, S.X. Lim, S.H.E. Hu, and B.C. Yiap, *Phytomedicine*, 2013;**20**;710-713.
33. Q. Liu, X. Meng, Y. Li, C.N. Zhao, G.Y Tang, H.B. Li. *International Journal of Molecular Sciences*, 2017;**18**(6);1283.
34. R. Dhiman, N. Aggarwal, K.R. Aneja and M. Kaur. *International Journal of Microbiology*, 2016;**4**;2016.
35. R. Kapilan. *Int J Res Granthaalayah*, 2015;**3**(10);57-60.
36. R. Tshabalala, A. Kabelinde, C.D.K. Tchatchouang, C.N. Ateba, and M.C. Manganyi. *Saudi Journal of Biological Sciences*, 2021;**28**(7);3855-3863.
37. S. Cho, L.M. Hiott, J.B. Barrett, E.A. McMillan, S.L. House, S.B. Humayoun, E.S. Jackson and C.R. Frye. *PLOS ONE*, 2018;**13**(5):0197005.
38. D.M. Dabbs, N. Mulders and I.A. Aksay. *Journal of Nanoparticle Research*, 2006;**8**:603-14.
39. Muhamad, N.D. Hassan, S.N. Mamat, N.M Nawi, W.A. Rashid and N.A. Tan. *Academic Press*, 2017;523-560.
40. S.F. Nabavi, A. Di Lorenzo, M. Izadi, E. Sobarzo-Sánchez, M. Daglia and S.M. Nabavi. *Nutrients*, 2015;**7**(9);7729-48.
41. S. PD'Souza, S.V. Chavannavar, B. Kanchanashri, S.B. Niveditha. *Journal of evidence-based complementary & alternative medicine*, 2017;**22**(4);1002-10.
42. S.A. Sulaiman. *Extraction of essential oil from Cinnamomum zeylanicum by various methods as a perfume oil* (Doctoral dissertation, UMP).
43. T.B. Wankhede. *Int Res J Sci Eng*, 2015;**3**;166-72.




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44. W.B. Jensen. *Journal of chemical education*, 2007;**84**(12);1913.
45. W. Guan, S. Li, R.S. Yan, C. Tang, *Food Chemistry*, 2007;**101**(4);1558-64.

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