Original Research Article

*In vitro*Evaluation of Antimicrobial Activity of *Syzigium aromaticum* (Clove)against Bacteria Isolated from Different Clinical Specimens in Shendi Town, Sudan

ABSTRACT

Background: Herbal medicinal products have been documented as a significant source for discovering new pharmaceutical molecules that have been used to treat serious diseases. It has been postulated that the geographical locations of the herbs affect the constituents of their essential oils and thus the degree of their antimicrobial action. *Syzigium aromaticum* (clove) is a traditional spice used as an antimicrobial agent and an alternative solution to increased antibiotic resistance by bacterial strains. ***Objective***: This study aimed to study the *in vitro* antimicrobial activity of different clove concentrations against bacteria isolated from clinical specimens. ***Methods***: This is a prospective cross-sectional study conducted in Shendi City, Sudan, from February to March 2023, at the Microbiology Laboratory, Faculty of Medical Laboratory Sciences. 50 samples were collected from urine and wounds, from which eight strains of pathogenic gram-positive and gram-negative bacteria were isolated and identified using gram stain and biochemical tests. Clove was tested using a 100%, 50%, 25%, and 12.5% concentration. ***Results***: Out of 50 clinical specimens, standard strains were confirmed as *S. aureus* 13 (26%), *S. epidermidis* 7 (14%), *E. faecalis* 1 (2%), *E. coli* 15 (30%), *Enterobacter* 3 (6%), *Citrobacter* 2 (4%), *K. pneumoniae* 4 (8%), and *P. aeruginosa* 5 (10%). Clove showed remarkable antimicrobial activity against all clinical isolates and standard strains. ***Conclusion***: The findings of this study showed that the aqueous extract of clove can exhibit high antimicrobial activity against all types of tested organisms, both clinical and standard. The findings indicate that, besides being safe and sensorial attractive, Clove has antimicrobial activity, making it an herbal antimicrobial agent.

Keywords:*Syzygium aromaticum*, oil, extract, clove,antimicrobial, antibiotic resistance, bacteria.

1. INTRODUCTION

The traditional medical approach, which relies on the application of herbal treatments, is still very much a part of the healthcare system. Because they are natural products with fewer side effects and greater efficacy than synthetic alternatives, therapeutic plants have gained more popularity in recent decades **[1,2].** Approximately 80% of people on the planet now receive the majority of their primary healthcare from traditional medicine **[3].** Many herbal plants have pharmacological properties that make them useful for food preservation, and other applications [26-19]. They also contain anti-inflammatory, antibacterial, spasmolytic, analgesic, and local anesthetic properties **[4,5].**Numerous plant species have been found to possess pharmacological properties linked to their phytoconstituents, which include tannins, alkaloids, glycosides, saponins, flavonoids, steroids, and terpenes **[4].** Herbal treatments are an important source for the discovery of new pharmaceutical compounds that have been utilized to treat serious illnesses up to this point. These newly discovered phytochemicals are thought to be an exceptional main compound in the hunt for novel and potent medications **[5].**Antimicrobial resistance has developed as one of the major urgent threats to public health causing serious issues for the successful prevention and treatment of persistent diseases, misusing and overusing different antibacterial agents in the health care setting as well as in the agricultural industry are considered the major reasons behind the emergence of antimicrobial resistance **[6].**The increasing incidence of drug-resistant pathogens raises an urgent need to identify and isolate new bioactive compounds from medicinal plants using standardized modern analytical procedures, The World Health Organization has stated that 80% of the developing world still benefits from the use of traditional medicines derived from medicinal plants. Extracts isolated from medicinal plants have also been reported to exhibit various biological activities such as antimicrobial, anti-inflammatory, and antioxidant activities**[7].***Syzigium aromaticum* (clove) is a traditional spice that has been used for food preservation and possesses various pharmacological activities that have been examined toward various pathogenic parasites and microorganisms, including pathogenic bacteria, Plasmodium, Babesia, Herpes simplex, and hepatitis C viruses. **[8].**Several reports documented the analgesic, antioxidant, anticancer, antiseptic, anti-depressant, antispasmodic, anti-inflammatory,

antiviral, antifungal, and antibacterial activity of eugenol against several pathogenic bacteria including methicillin-resistant *Staphylococcus epidermidis* and *S. aureus***[8,9].**In the world, clove treats many diseases such as asthma, dental caries, respiratory disorders, headaches, and sore throats. It is also used in traditional medicine to treat digestive disorders such as dyspepsia, gastritis, and diarrhea**[9].**In Sudan, the Arabs knew clove as scornful and it has been used to flavor cooking, in the bakery industries, and in the production of sauces and pickles, also it has many therapeutic uses, itrelieves pain, controls nausea and vomiting, improves digestion and slows down the symptoms of cholera if it boils in water. Also, chewing clove relieves the irritation of the throat and stops coughing in pharyngitis, also used in dentistry for treating minor oral wounds as an analgesic in painful and infective diseases of the oral cavity**[10].**This present study aimed to assess the antimicrobial activity of *Syzigium aromaticum* (Clove)against bacteria isolated from different clinical specimens in Shendi Town, Sudan.

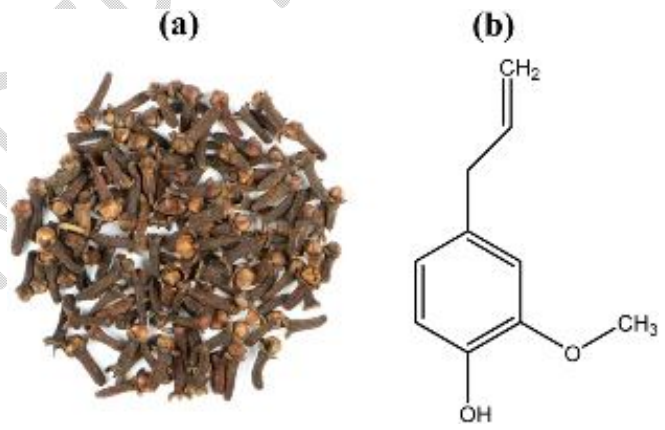


Fig.1.*Syzygium aromaticum* (clove): (a) physical appearance and (b) chemical structure of eugenol **[25].**

2. MATERIALS AND METHODS

2.1 Study design

A descriptive cross-sectional community and hospital-based study. The samples were distributed between different hospitals and clinical centers located in Shendi locality, River Nile State, Sudan Shendi is a town in Northern Sudan, situated on the east bank of the Nile (150 km) northeast of Khartoum. Shendi is also about 45 kilometers southwest of the ancient city of Meroe.

2.2 Samples Collection

Several 50 urine and wound swab specimens under aseptic status were collected (n = 50) at Shendi town, River Nile, Sudan.

2.3 Bacterial Isolation and Identification

The isolated bacteria were identified by colony morphology, Gram stain, and biochemical tests. Full identification was done by using the Gram staining technique and specific confirmatory biochemical tests. After identification, stock cultures were made and then kept in the refrigerator at the optimum temperature.

2.4 Collection and Preparation of *Syzygium aromaticum*(Clove)

Specimen of clove was obtained from a local supermarket in Shendi city "Ahfad Alteman atara". The dried Clove sample was cleaned from dust and then crushed to a coarse powder, one hundred grams of clove powder were dissolved in one liter of boiled D.W and incubated for 24 hours then the mixture was filtered using filter paper and then preserved in a refrigerator until use **[11].**

2.4.1 Concentrations of *Syzygium aromaticum* (Clove)

Syzygium aromaticum was used in different concentrations (100%, 50%, 25, and 12.5%) against isolated organisms.

2.4.1 Preparation of standard bacterial suspension

Clinical isolates were isolated from different samples in a sterile slope of nutrients and standard bacteria were brought from the microbiology department of the National Institute for Research, Ten ml of normal saline were distributed in test tubes and sterilized in an autoclave at121oC for 15 minutes. A loop full of purified bacteria was inoculated in sterile normal saline. The inoculum density was compared with the McFarland standard solution.

2.5 Cup-plate agar diffusion method

The agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts.Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism, and emulsify in 3–4 ml of sterile physiological saline or nutrient broth. In a good light, match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities, it is easier to compare against a printed card or sheet of paper. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate at approximately 60 to ensure even distribution. With the petri dish lid in place, allow 3–5 minutes for the surface of the agar to dry. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer; clove extract is introduced into the well at the desired concentration, and agar plates are incubated under suitable conditions. By using a ruler on the underside of the plate, measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts **[12].**

2.6 Data Collection and Analysis

A self-administered questionnaire was used and supported with coding numbers to facilitate the sorting of data. The data were entered, checked, and analyzed using Microsoft Excel 2007. The final results were presented as frequencies and percentages.

3. RESULTS

A total of 50 patients with symptoms of urinary tract infection and wound infection were enrolled in this study during the period from February to March 2023. Among the studied population of fifty patients, there were 22 (44% males) and 28 (55% females) (Table **1).** Among the different clinical samples tested, there were 38 urine samples and 12 wound swabs **(Table 2).** Clinical isolates were tested as follows: 13 (26%) *S. aureus*, 7 (14%) *S. epidermidis*, 1 (2%) E. *fecalis*, 15 (30%) E. coli, 4 (8%) *K. pneumoniae*, 3 (6%) *Enterobacter*, 2 (4%) *Citrobacter*, and 5 (10%) *P. aeruginosa***(Table 3).** Among gram-positive bacteria, there were 100% of organisms sensitive to both stock clove and (50% v/v) dilution and no resistance; there were also 76% sensitive to (25% v/v) clove and

only24% resistance, and there were 71% sensitive to 12.5% v/v) clove and only 29% resistance **(Table 4).** Among gram-negative bacteria, 100% of organisms were sensitive to stock clove 93% were sensitive to 50% (v/v) clove and 7% resistance; 52% were sensitive to 25% (v/v) clove and 48% resistance, and 24% were sensitive to 12.5% (v/v) clove and 76% resistance **(Table 5).** Among gram-positive bacteria, the highest mean diameter of the growth inhibition zone in mm (MDIZ) of clove for *S. aureus* was 19.6 mm at a concentration of 100%, while the lowest (MDIZ) was 11.5 mm at a concentration of 12.5%, The highest (MDIZ) of *S. epidermidis* was 18 mm at a concentration of 100%, while the lowest (MDIZ) was 11.1 mm at a concentration of 12.5%, The highest MDIZ of *E. fecalis* was 18 mm at a concentration of 100%, while the lowest was 7 mm at a concentration of 12.5% **(Table 6).** Among gram-negative bacteria, the highest (MDIZ) of E. coli was 18.5 mm at a concentration of 100% while the lowest (MDIZ) was 7.6 mm at a concentration of 12.5%; the highest (MDIZ) of *K. pneumoniae* was 16 mm at a concentration of 100% while the lowest (MDIZ) was 7 mm at a concentration of 12.5%; the highest (MDIZ) of *Enterobacter* was 18.3 mm at a concentration of 100% while the lowest (MDIZ) was 9.7 mm at a concentration of 12.5%; the highest (MDIZ) of Citrobacter was 17.5 mm at concentration 100% while the lowest (MDIZ) was 7 mm at concentration 12.5%; the highest (MDIZ) of *P. aeruginosa* was 15.8 mm at concentration 100% while the lowest (MDIZ) was 9.2 mm at concentration 12.5% **(Table 7).** Among gram-positive bacteria tested in this study, the highest (MDIZ) was 18.5 mm at a concentration of 100% and the lowest (MDIZ) was 9.9 mm at a concentration of 12.5%, while in gram-negative bacteria, the highest (MDIZ) was 17.2 mm a concentration of 100% and the lowest (MDIZ) was 8.1 mm a concentration of 12.5% **(Table 8).** The water extract of clove presented variable activity against standard strains *S. aureus* ATCC 25923, followed by *K. pneumoniae* ATCC53657, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *E. fecalis*, Citrobacter and *Enterobacter***(Table 9).**

Table 1. The distribution of clinical specimens according to the gender.

Gender	Frequency	Percentage
Male	22	44%
Female	28	56%
Total	**50**	**100%**

Table 2. The distribution of clinical specimens according to the sample Type.

Type of sample	Frequency	Percentage
Urine	38	76%
Wound swab	12	24%
Total	**50**	**100%**

Table 3. The frequency and percentage of isolated organisms.

Isolate	Frequency	Percentage
S.aureus	13	26%
S.epidermidis	7	14%
E.fecalis	1	2%
E.coli	15	30%
K.pneumoniae	4	8%
Entereobacter	3	6%
Citrobacter	2	4%
P.aeruginosa	5	10%
Total	**50**	**100%**

Table 4. The sensitivity of gram-positive bacteria to differentconcentrations of clove

Pathogens	Concentration of clove							
	100%		50%		25%		12.5%	
	S	R	S	R	S	R	S	R
S.areus	13	0	13	0	11	2	10	3
S.epedermidis	7	0	7	0	5	2	5	2
E.fecalis	1	0	1	0	0	1	0	1
Percentage	**100%**	**0%**	**100%**	**0%**	**76%**	**24%**	**71%**	**29%**

Table 5. The sensitivity of gram-negative bacteria to differentconcentrations of clove

Pathogens	Concentration of clove							
	100%		50%		25%		12.5%	
	S	R	S	R	S	R	S	R
E.coli	15	0	14	1	8	7	3	12
K.pneumoniae	4	0	4	0	2	2	0	4
Enterobacter	3	0	3	0	3	0	2	1
Citrobacter	2	0	2	0	0	2	0	2
P.areuginosa	5	0	4	1	2	3	2	3
Percentage	**100%**	**0%**	**93%**	**7%**	**52%**	**48%**	**24%**	**76%**

Table 6. The mean diameter of growth inhibition zone (MDIZ) in millimeters (mm) of clove against gram-positive pathogen

Pathogens	Concentration of clove			
	100%	50%	25%	12.5%
S.areus	19.6	16.6	13	11.5
S.epedermidis	18	15	11.5	11.1
E.fecalis	18	15	7	7

Table 7. The mean diameter of growth inhibition zone (MDIZ) in millimeters(mm) of clove against gram-negative bacteria

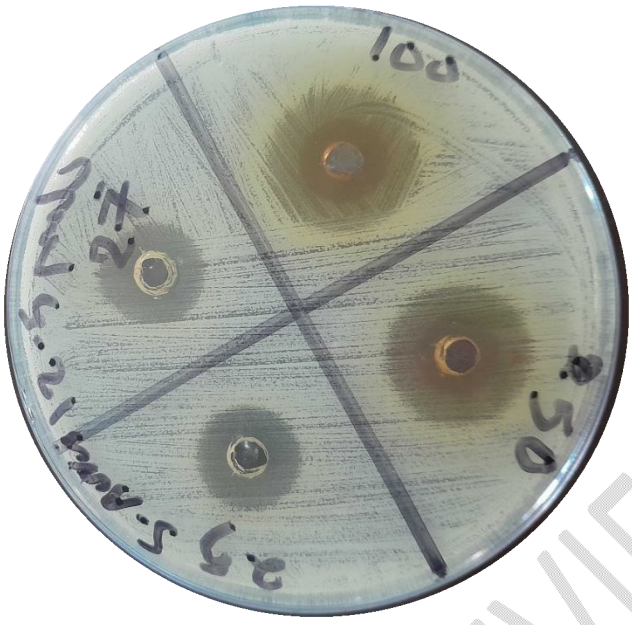
Pathogens	Concentration of clove			
	100%	50%	25%	12.5%
E.coli	18.5	14.9	10.1	7.6
K.pneumoniae	16	14	10.2	7
Enterobacter	18.3	16	14	9.7
Citrobacter	17.5	14.5	7	7
P.aeruginosa	15.8	13.8	10.6	9.2

Table 8.The comparison between the mean diameter of growth inhibition zone (MDIZ) in millimeters (mm) of clove against gram-positive and negative bacteria

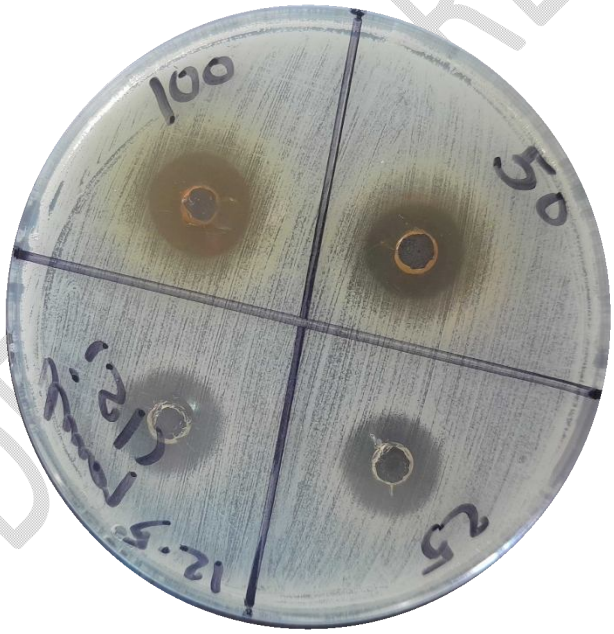
Pathogens	Concentration of clove			
	100%	50%	25%	12.5%
Gram-positive	18.5	15.5	10.5	9.9
Gram-negative	17.2	14.6	10.4	8.1

Table 9. Antimicrobial activity of clove against standard organisms.

STD organism	100%	50%	25%	12.5%
S.aureus	S	S	S	S
S.epidermidis	S	S	S	S
E.fecalis	S	S	S	R
E.coli	S	S	R	R
K. pneumonia	S	S	S	S
Enterobacter	S	S	S	S
Citrobacter	S	S	R	R
P.aeruginosa	S	S	S	R



Picture1. Show the activity of *Syzygium aromaticum* against *S. aureus*.



Picture 2. Show the activity of *Syzygium aromaticum* against *E.coli* ATCC 25922.

4. DISCUSSION

Traditionally, cloves have been used for centuries in the treatment of vomiting, flatulence, nausea, liver, bowel, and stomach disorders; and as a stimulant for the nerves. In tropical Asia, cloves have been documented to relieve different microorganisms such

as scabies, cholera, malaria, and tuberculosis. As well, in America, clove has been traditionally used to inhibit food-borne pathogens to treat viruses, worms, candida, and different bacterial and protozoan infections**[13].**Misusing and overusing different antibacterial agents in the healthcare setting as well as in the agricultural industry are considered the major reasons for developing antimicrobial resistance, which threatens public health**[6].**The increasing incidence of drug-resistant pathogens raises an urgent need to identify and isolate new bioactive compounds from medicinal plants and extracts isolated from them have been reported to exhibit various biological activities such as antimicrobial, anti-inflammatory, and antioxidant activities **[7].**The antibacterial activity of clove (*syzigum aromaticum*) is due to the presence of major active compounds, eugenol and β-caryophyllene**[14].**In this study, we attempted to assess the antimicrobial activity of clove in different concentrations against clinically isolated bacteria. The antibacterial activity was tested using the well diffusion assay and the clove samples were tested at 100%, 50%, 25%, and 12.5% concentrations. Clove showed the highest activity against *S. aureus* followed by *E.coli,* and the lowest activity against *Citrobacter* followed by *E. faecalis.* The results indicated that the most effective concentration was 100% and the effect of clove was decreased dramatically when the concentration of clove was decreased and the vase reversed. It also showed that the clove was affected by both gram-positive and gram-negative bacteria, but their effect on gram-positive bacteria was to a greater extent than that of gram-negative bacteria, these results agreed with that obtained by José Nabor Haro-González**[15].**In this study, the aqueous extract of clove (*Syzigium aromaticum*)showed remarkable antimicrobial activity against standard and clinical isolates of *S. aureus, S. epidermidis, E.faecalis, E. coli, Ps. aeruginosa, K. pneumoniae, Enterobacter and Citrobacter.* These findings are in agreement with several studies cited below. Gaber El-Saber Batihaand his colleagues found that eugenol was active against several pathogenic bacteria including *methicillin-resistant Staphylococcus aureus* and *S.epidermidis***[8].**In 2008 G. A. Ayoola and his colleagues found that clove showed antimicrobial activity against *Staphylococcus aureus* ATCC 25923 and some Gram-negative bacteria (*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae, Salmonella paratyphi, Citrobacter spp, and Enterobacter cloacae*) **[16].**Deepanjeet Kaur and Kaushal K Chandrul found that the aqueous extract of clove showed a complete bactericidal effect against all the food-borne pathogens tested*Escherichia coli (E. coli),*

Staphylococcus aureus and *Bacillus cereus* **[17].**This alsocomes in line with a study done by Simiat Olanike Jimoh1, Lateefah Adelanke Arowolo1 and Kazeem Adelani Alabi who found that clove was active against some microorganismsincluding*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa***[18].**In this study, clove showed a strong effect against *E.coli* with an inhibition zone of 18.5mm which is greater than the inhibition zone obtained by S.A. Burt and R.D. Reinders who found that the clove bud extract showed strong antimicrobial activity against *E.coli* with an inhibition zone (15.7 mm)**[19].**Also in this study *S. aureus* was the most susceptible of all the tested bacteria showing a very strong inhibitory activity,this result is inagreement with the result obtained by MiroslavaKaˇcániová and his colleagues**[9].**Also, this study comes in line with the result obtained by Kamel Chaieb and his colleagues who found that clove essential oil produces a clear zone of inhibition against five strains of *S. epidermidis* (reference strains *S. epidermidis* CIP106510, E13, S27, S23 and S38) **[20].**Also, this study comes in line with the result obtained by Anshul Shah and his colleagues who found that clove had inhibitory action against *enterococcus faecalis***[21].**Also this study agreement with the study done by Baydaa Hameed Abdullah, Suhad Faisal Hatem, and Widad Jumaa who found that clove had a strong antimicrobial effect against two standard strains, *Staphylococcus aureus*ATCC29213*and Pseudomonas aeruginosa*ATCC27853 **[22].**Similarly, the recent study contravenes the study of Vanessa Lee Rosario and his colleagues who found that the effect of clove extract on *S. epidermidis* is greater than the effect on *S. aureus***[23].**Also, it contradicts the study of L. Nuñez and M. D' Aquino who found that *E.coli*was the most sensitive organism to clove compared to other tested organisms (*S. aureus* and *P. areuginosa*)**[24].**

5. CONCLUSION

In this study, it was concluded that an aqueous extract of clove (*syzigium aromaticum*) possesses a remarkable antimicrobial effect on gram-positive and gram-negative bacteria. Based on this research, the findings showed that clove in its most concentrated forms was very effective against all pathogenic bacteria included in this research. Additionally, the antibacterial activity of clove in concentrations other than 100% depended on the type of bacterial species, which means the antibacterial effect can be changed from one strain to another. We believe this investigation together with previous studies provided support for the antimicrobial properties of clove. Clove can be a solution to the antibiotic resistance

problem can provide a new source of alternative drugs for antibiotics and can help in avoiding and minimizing the side effects of these antibiotics.

CONSENT

The patient’s written consent has been collected.

ETHICAL APPROVAL

Permission was given by the College Ethical Committee of Shendi University and Hospitals. Participators have been noticed and no coercion of any sort has been done and any information that may disclose the participator's identity was not kept in consideration.

Disclaimer (Artificial intelligence)

As a result, the Author (s) declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

References:

1. Abushouk, A.I.; Negida, A.; Ahmed, H.; Abdel-Daim, M.M. Neuroprotective mechanisms of plant extracts against MPTP induced neurotoxicity: Future applications in Parkinson’s disease. Biomed. Pharmacother. 2017, 85, 635–645. [Google Scholar] [CrossRef]
2. Abushouk, A.I.; Ismail, A.; Salem, A.M.A.; Afifi, A.M.; Abdel-Daim, M.M. Cardioprotective mechanisms of phytochemicals against doxorubicin-induced cardiotoxicity. Biomed. Pharmacother. 2017, 90, 935–946. [Google Scholar] [CrossRef] [PubMed]
3. Ekor, M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 2014, 4, 177. [Google Scholar] [CrossRef] [PubMed] [Green Version]
4. Batiha, G.E.S.; Beshbishy, A.A.; Tayebwa, D.S.; Shaheen, M.H.; Yokoyama, N.; Igarashi, I. Inhibitory effects of Uncaria tomentosa bark, Myrtus communis roots, Origanum vulgare leaves, and Cuminum cyminum seeds extracts against the growth of Babesia and Theileria in vitro. Jap. J. Vet. Parasitol. 2018, 17, 1–13.

[Google Scholar]

5. Beshbishy, A.M.; Batiha, G.E.S.; Adeyemi, O.S.; Yokoyama, N.; Igarashi, I. Inhibitory effects of methanolic Olea europaea and acetonic Acacia laeta on the growth of Babesia and Theileria. Asian Pac. J. Trop. Med. 2019, 12, 425–434. [Google Scholar]
6. Dadgostar P. Antimicrobial Resistance: Implications and Costs. Infect Drug Resist. 2019 Dec 20;12:3903-3910. doi: 10.2147/IDR.S234610. PMID: 31908502; PMCID: PMC6929930.
7. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms. 2021 Sep 27;9(10):2041. doi: 10.3390/microorganisms9102041. PMID: 34683362; PMCID: PMC8541629.
8. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. Biomolecules. 2020 Jan 30;10(2):202. doi: 10.3390/biom10020202. PMID: 32019140; PMCID: PMC7072209.
9. Kačániová M, Galovičová L, Borotová P, Valková V, Ďúranová H, Kowalczewski PŁ, Said-Al Ahl HAH, Hikal WM, Vukic M, Savitskaya T, Grinshpan D, Vukovic NL. Chemical Composition, *In Vitro* and *In Situ* Antimicrobial and Antibiofilm Activities of *Syzygium aromaticum* (Clove) Essential Oil. Plants (Basel). 2021 Oct 15;10(10):2185. doi: 10.3390/plants10102185. PMID: 34685994; PMCID: PMC8538430.
10. Abdel Moneim E, Iman MO, AL Amin A. Nutritive value of clove (Syzygium aromaticum) and detection of antimicrobial effect of its bud oil. Res. J. of Microb. 2007;2:266-71.
11. Rahim ZH, Khan HB. Comparative studies on the effect of crude aqueous (CA) and solvent (CM) extracts of clove on the cariogenic properties of Streptococcus mutans. J Oral Sci. 2006 Sep;48(3):117-23. doi: 10.2334/josnusd.48.117. PMID: 17023743.
12. Valgas C, Souza SM, Smânia EF, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. Brazilian journal of microbiology.

2007;38:369-80.

13. Bhowmik, D.; Kumar, K.S.; Yadav, A.; Srivastava, S.; Paswan, S.; Dutta, A.S. Recent trends in Indian traditional herbs Syzygium aromaticum and its health benefits. J. Pharmaco. Phytochem. 2012, 1, 13–23. [Google Scholar].
14. Hiwandika N, Sudrajat SE, Rahayu I. Antibacterial and antifungal activity of clove extract (*Syzygium Aromaticum*). Eureka Herba Indonesia. 2021 Apr 26;2(2):86-94.
15. Haro-González JN, Castillo-Herrera GA, Martínez-Velázquez M, Espinosa-Andrews H. Clove essential oil (Syzygium aromaticum L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. Molecules. 2021 Oct 22;26(21):6387.
16. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HA, Odugbemi TO. Chemical analysis and antimicrobial activity of the essential oil of Syzigium aromaticum (clove). African Journal of Microbiology Research. 2008 Jul 1;2(7):162-6.
17. Kaur D, Chandrul KK. Syzygium aromaticum L.(Clove): A vital herbal drug used in periodontal disease. Indian Journal of Pharmaceutical and Biological Research. 2017 Jun 30;5(02):45-51.
18. Jimoh SO, Arowolo LA, Alabi KA. Phytochemical screening and antimicrobial evaluation of Syzygium aromaticum extract and essential oil. Int. J. Curr. Microbiol. Appl. Sci. 2017;6:4557-67.
19. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against Escherichia coli O157:H7. Lett Appl Microbiol. 2003;36(3):162-7. doi: 10.1046/j.1472-765x.2003.01285.x. PMID: 12581376.
20. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB, Rouabhia M, Mahdouani K, Bakhrouf A. The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review. Phytother Res. 2007 Jun;21(6):501-6. doi: 10.1002/ptr.2124. PMID: 17380552.
21. Shah A, Jani M, Shah H, Chaudhary N, Shah A. Antimicrobial effect of Clove oil (Laung) extract on Enterococcus faecalis. Journal of Advanced Oral Research. 2014 Sep;5(3):36-8.
22. Abdullah BH, Hatem SF, Jumaa W. A comparative study of the antibacterial

activity of clove and rosemary essential oils on multidrug-resistant bacteria. Pharmaceutical and Biosciences Journal. 2015 Feb 27:18-22.

23. Rosarior VL, Lim PS, Wong WK, Yue CS, Yam HC, Tan SA. Antioxidant-rich clove extract, a strong antimicrobial agent against urinary tract infections-causing bacteria in vitro. Tropical life sciences research. 2021 Jun;32(2):45.
24. Nuñez L, Aquino MD. Microbicide activity of clove essential oil (Eugenia caryophyllata). Braz J Microbiol. 2012 Oct;43(4):1255-60. doi: 10.1590/S1517-83822012000400003. Epub 2012 Jun 1. PMID: 24031950; PMCID: PMC3769004.
25. Yameen M, Asghar F, Adeel S, Haider MZ, Özomay M, Aftab M, Mia R. Enhancing wool dyeing with clove bud (*Syzygium aromaticum*) based natural dye via microwave treatment using a central composite design. Sci Prog. 2023 Oct-Dec;106(4):368504231215593. doi: 10.1177/00368504231215593. PMID: 37993993; PMCID: PMC10666708.
26. Michael Ikon, Grace, Victor Nelson Abasiubong, and Chijioke Patrick Amadi. 2017. "Antimicrobial Activity of Garlic Extract on Organisms Isolated from Tomato Rot". *Journal of Advances in Microbiology* 7 (2):1-10. https://doi.org/10.9734/JAMB/2017/37590.
27. O. Ihuma, J., T. D. Malgwi, and M. H. Matthew. 2022. "In Vitro Antimicrobial Activity of Phyllanthus Urinaria (Linnaeus, Phyllanthaceae) Leaves Against Staphylococcus Aureus and Pseudomonas Aeruginosa Isolated from Wounds". *Asian Journal of Biology* 15 (2):38-46. https://doi.org/10.9734/ajob/2022/v15i230237.
28. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016 Apr 1;6(2):71-9.
29. Savluchinske-Feio S, Curto MJ, Gigante B, Roseiro JC. Antimicrobial activity of resin acid derivatives. Applied microbiology and Biotechnology. 2006 Sep;72:430-6.