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Research Paper

A comparative study between the effect of aqueous and alcoholic extracts of cloves on inhibiting the growth of some gram-positive and gram-negative bacteria

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ABSTRACT

Background: Clove (Syzygium aromaticum) shows broad spectrum antibacterial effects, it works on both Gram-positive and Gram-negative microorganisms too. Because of this, its usefulness as a natural antimicrobial is worth a more careful, step by step study using consistent solvent extraction procedures… like the standardized ones.
Objective: The aim was to see how strongly aqueous and alcoholic clove extracts can slow, or inhibit, bacterial growth when the concentration is set at 5% and 10%. Focus was on Gram-positive as well as Gram-negative bacteria.
Methods: In total, 80 bacterial samples were taken. They included Gram-positive and Gram-negative isolates, plus another 20 control samples. Clove was processed into two kinds of extracts: aqueous, meaning water based, and alcoholic, meaning 96% ethanol based. Each extract type was made at 5% and at 10%. For the Gram-positive side, 20 samples were exposed to each extract type at both concentrations. For the Gram-negative side, 40 samples were treated the same way, using aqueous and alcoholic extracts at 5% and 10%. After treatment, antibacterial effects were checked by observing bacterial growth inhibition across all groups.
Results: Both aqueous and alcoholic clove extracts had clear inhibitory action against Gram-positive and Gram-negative bacteria at 5% and 10%. In general, the 10% concentration was more effective than 5% for both bacterial groups, and this held up for both solvent types. Also, there was no obvious, standout advantage of aqueous over alcoholic, or vice versa.
Conclusion: Overall, clove plant extracts can produce measurable antimicrobial activity across a wide set of bacteria, and the effect is concentration dependent. These results also hint that clove-derived compounds could be considered later, as potential antibiotic alternatives for handling microbial infections, although more work is still needed, both in the lab and in clinical settings.

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1. INTRODUCTION

Cloves are a small, evergreen, perennial tree. It is a type of beautiful spice known for its wonderful flavor, which made it used as a type of spice. It has a conical shape with a four-parted flower. It belongs to the clove family. Its flowers are picked twice a year and dried. Its buds turn from red to brown after drying and have a pleasant aroma. It has a strong aroma and is one of the oldest and most famous spices. Its shape resembles a nail and is its most widely used part. The clove tree is classified within the Asian family, which is known for its medium size, as its length ranges between (8 to 12) meters.

The clove tree brings many benefits. Its origins go back to eastern Indonesia and it grows in coastal areas that are about 200 meters above sea level. The clove tree's flower buds begin to grow 4 years after planting, as they are picked either by hand or by chemical methods, thus forming the commercial part of the clove tree. Regarding the names of cloves, in the Arab world, cloves are known as nails due to the resemblance between them and nails. They are also called cloves.

It was used for its strong aromatic smell, and cloves were used for centuries in various fields. Different types of spices, such as thyme, cinnamon, and mint, were known for their ability to act as food preservatives, and were considered medicinal substances due to their antioxidant and antimicrobial effects. Cloves contain 20% volatile oil and eugenol oil, which It has an anesthetic property, in addition to several minerals such as phosphorus, sodium, iron, magnesium, potassium, calcium, manganese, and some acids such as hydrochloric acid. It also contains vitamins (C and K), calcium, magnesium, and fiber, and it contains eugenol, which helps relieve pain and kill germs. Recently, these and other properties of spices in general and cloves in particular have been proven, as it was found to have an anti-bacterial and anti-fungal effect and properties that make them anti-cancer, and cloves specifically were found to contain effective and powerful antioxidants in addition to their anti-microbial activity compared to other spices [1].

As for the benefits of cloves, they contain fiber, vitamins, and minerals that make adding them to foods beneficial for health, whether in their whole or powdered form, in addition to the fact that they contain important elements to maintain the health of the body, as they are low in calories [2].

One of the health benefits of cloves is the prevention of constipation, as cloves contain fibers that help prevent constipation and strengthen the immune system. Vitamin C in cloves can help strengthen the immune system and maintain bone health, as it contains manganese, which It is involved in bone formation and plays an important role in maintaining bone health Consumption of manganese supplements increases bone density and growth, and clove extract improves indicators of osteoporosis and increases bone density and strength. Also, the fact that clove powder contains an amount of calcium adds another benefit to it. It should be noted that millions of elderly people in the world suffer from low bone density, which is a condition. A disease that results in osteoporosis, thus increasing the risk of fractures and ruptures, alleviating stomach ulcers, Clove extract also contributes to the treatment of stomach ulcers, and helps regulate blood sugar levels, as insulin is a hormone responsible for transferring sugar from the blood to the cells of the human body. Therefore, the proper functioning of insulin is necessary to maintain regular blood sugar levels. It has been found through some studies that cloves contain compounds that help enhance insulin production, It has the potential to improve liver health because cloves contain compounds that help enhance liver health and reduce inflammation and oxidative stress. This compound helps prevent the appearance of signs of liver cirrhosis. The ability of eugenol and cloves to protect the liver is still limited, and taking eugenol supplements for one week may reduce the

levels of an enzyme called (GST) is an enzyme that contributes to the removal of toxins that are often an indicator of liver disease. If it is taken in high quantities, ranging from (5 to 10) milliliters of clove oil, serious liver damage may occur. Caution must be taken and not to take a large amount of clove oil [3], [4].

Regarding the harmful effects of cloves, clove oil or ointments containing clove flower are safe when used on the skin. However, repeated use of clove oil in the mouth or on the gums can sometimes cause damage to the gums, nerves, skin, and mucous membranes. Injecting clove oil into the veins is considered a risk. Unsafe behaviors can cause side effects such as breathing problems and lung diseases. Dried cloves may cause mouth sensitivity and irritation as well as damage to dental tissue.

There are also some groups that warn against consuming cloves, as they can cause serious side effects in children, such as seizures, liver damage, or fluid imbalance. Cloves are considered safe when taken through food, but there is not enough reliable information about the safety of taking them. Cloves are used in medical doses, so it is not recommended to take cloves during pregnancy or breastfeeding.

Regarding clove oil, it contains the compound eugenol, which in turn helps slow the blood clotting process. Therefore, there are concerns that taking clove oil may cause bleeding in people who suffer from bleeding disorders, and cloves contain the compound eugenol, which slows blood clotting. As for the medical benefits of cloves in alternative medicine, it purifies the blood, treats peptic ulcers, is useful in cases of nausea and dizziness, and is used to treat throat pain because it contains volatile oils that are effective in relieving pain, works to stimulate blood circulation, helps focus, prevents insomnia and depression, and contributes to strengthening the heart and liver, and also strengthens eyesight. By drinking it as a hot drink, it is used to treat digestive problems such as bloating, skin infections, and insect bites. It is useful in cases of colds, bronchitis, and blocked sinuses. It is also useful in treating coughs and asthma [5].

The benefits of cloves for the teeth: It helps in relieving and anesthetizing pain because it contains a compound called eugenol, which is a chemical compound that is widely used as an anesthetic and pain reliever. Using clove oil and clove buds has a greater benefit for the teeth and removes pain from them. How to use: Put a few clove heads in the mouth until they become soft. by the action of saliva, then chew them on the molars to extract the oil contained in the cloves to relieve pain. If the person is not able to chew the cloves, he can use (powder). It should be noted that the compound eugenol may cause poisoning if ingested. Therefore, this treatment should not be used for children who are more susceptible and allergy to oil.

The benefits of cloves for pregnant women: It contains many important minerals such as manganese, phosphorus, and sodium, which help in promoting the overall development of the fetus. It contains dietary fiber, which helps in reducing digestive system problems during pregnancy, such as constipation, which often accompanies pregnancy. It is an excellent source of vitamins (K) and (C) which help maintain the mother's health during pregnancy. Cloves contain omega-3 acids that are beneficial for the development of the fetus's nervous system. It contains calcium and phosphorus, which help in the growth of bones and teeth in children. One of its benefits is that it is included in the eyeliner that limits vision, removes blurring, and clears vision, removing blurred vision.

As for the benefits of cloves for the body, it soothes abdominal pain by drinking clove tea, which contains many benefits, including eliminating gases, helping in digestion, killing harmful bacteria in the stomach, treating joint pain, and removing blurred vision.

It lowers blood pressure, produces urine, and eliminates kidney and bladder infections. It does not contain the caffeine that coffee contains, which has a stimulant effect. Cloves are a safe plant, provided they are consumed in moderation. Cloves can be added to tea and coffee, and cloves are used to strengthen the brain [6].

2. RELATED WORK

The original homeland of cloves is the Moluccas Islands in Indonesia, located near the equator and south of the Philippines. Today, cloves are widely grown in several countries, including Brazil,

Madagascar, and the Andes Mountains. Indonesia, India, Malaysia, Sri Lanka, and Tanzania, especially the island of Zanzibar, are among the most important countries that produce cloves on a commercial level. The spices on those islands were the first thing that drew the attention of merchants to the Indonesia region. The most important islands in the region were Ternate, Tidore, Halmahera, Ambon, and the Banda Islands. The Portuguese were the first to bring cloves to Ternate and Tidore in 1513 AD. They later established commercial colonies on both islands in in 1579 AD, the British explorer Sir Francis Drake visited the Spice Islands and bought a small number of cloves from Ternate. In 1600 AD, Dutch ships arrived in the islands in search of spices. In 1605 AD, under the Dutch monopoly, the cultivation of cloves and nutmeg was only on the Banda and Ambon Islands, and the Spice Islands were eventually annexed. To the Dutch East Indies which is now Indonesia.

Nutrition and slimming expert Keri Glassman say in her book (The Oxygen Diet) that eating foods rich in antioxidants helps in losing weight, that is, helps in slimming. Glassman calls for eating foods that contain the most antioxidants in order to lose a few kilograms of weight and achieve good health. Glassman said the benefits of cloves for the skin, thanks to the presence of eugenol, a natural compound that acts as a skin cleanser, cloves can be used to treat pimples and acne on the skin. The effect of cloves on the bacteria that cause acne [7].

3. METHODOLOGY

3.1. Practical Method for Preparing Aqueous Extract of Cloves

Water is one of the most common solvents used to extract active ingredients from plants. Dry clove powder is prepared after grinding it, then 50 grams of this powder is weighed and added to 1000 ml of distilled water in a clean glass beaker and left for a full day at laboratory temperature. After that, we filter the mixture to obtain the clear odor using pieces of clean gauze. We take this. Clear and filtered using filter paper. The resulting filtration process is placed in glass dishes at laboratory temperature for several days until the drying process is complete. We repeat this experiment several times to obtain the appropriate weight for conducting the experiment. The obtained powder is placed in the refrigerator at a temperature of 4 degrees Celsius for the purpose of Use it at the appropriate time to conduct the experiment as stated in Khudair [8]. "Preparation of aqueous extract of clove plant," 2021.

3.2. Practical Method for Preparing Alcoholic Extract of Cloves

The method of preparing the alcoholic extract includes the same steps followed in preparing the aqueous extract, but this method does not include the use of water. Rather, ethanol at a concentration of 96% is used to obtain the alcoholic extract. as identified in Hudayr [9].

3.3. How to Prepare 5% and 10% Concentrations of Aqueous and Alcoholic Extracts

1 gram of plant extract is weighed and then dissolved in 100 ml of distilled water to obtain a 5% concentration. To obtain a 10% concentration of this used extract, 0.5 of the plant extract is weighed and then dissolved in 100%. ml of distilled water. As for the alcoholic extract, the same method is followed that was used to prepare the aqueous extract, but dissolution is done in ethyl alcohol to obtain a concentration of 5% and 10% [10].

3.4. Testing the Effectiveness of the Aqueous and Alcoholic Extract on Bacteria

3.4.1. Agar Dilution Method

- Prepare Muller-Hinton agar medium and pour it into dishes, leave it to solidify, then make small holes in it using a cork drill, at wide intervals, 3-4 holes per dish.
- Each plate is planted with a specific type of bacteria by spreading or densely planning to fill the surface of the cake, especially around the holes and in a perpendicular manner.
- Add equal quantities of plant extracts (a few drops) into each hole, and make sure that the material does not spill on the surface of the medium (around the holes).

- The dishes are left to dry as the liquids spread inside the holes by spreading within the culture medium in the areas surrounding the holes. The dishes can then be incubated in the incubator upside down.
- After the end of the incubation period, the results are read. The positive result is the appearance of a halo of inhibition (a transparent area around the holes), the diameters of which are measured and the name of the extract and bacteria grown in the dish is recorded. The negative result is the appearance of growth around the holes and the destruction of the inhibition aura [11].

3.4.2. The Disc Method

This method uses discs of filter paper with a diameter of 5 mm. These discs are taken and saturated with aqueous and alcoholic extracts, each separately at concentrations of 5% and 10%, then left to dry at laboratory temperature for half an hour. A set of filter paper discs of equal diameters for each type of the extracts used in order to be saturated with the required concentration for each extract. These discs were then transferred to the surface of the cultured dish. Bacterial isolates using tweezers, then incubated for 24 hours. At 37°C, the results were recorded by measuring the diameter of the growth inhibition zones. Bacteria around each disc [12].

4. RESULTS AND DISCUSSION

The results in Table 1 showed that the effect of the aqueous extract of cloves at a concentration of 10% on Gram-positive bacteria in the samples taken in this study is more than at a concentration of 5%.

Table 1. Zone of Inhibition Values (MM) of Different Concentrations of Aqueous Extracts of Cloves of Some Gram-Positive Isolates

No. of Sample	Concentration of Aqueous Extract 5%	Concentration of Aqueous Extract 10%
1	15	19
2	17	20
3	15	12
4	14	13
5	15	12
6	16	16
7	10	12
8	12	15
9	12	16
10	15	20

Table 2 shows the effect of the alcoholic extract of the clove plant at a concentration of 10% more than its effect at a concentration of 5% on gram-positive bacteria in the samples under study.

Table 2. Zone of Inhibition Values (MM) of Different Concentrations of Alcoholic Extracts of Cloves of Some Gram-Positive Isolates

No. of Sample	Concentration of Alcoholic Extract 5%	Concentration of Alcoholic Extract 10%
1	15	15
2	15	12
3	15	19
4	19	18
5	19	12
6	12	12
7	16	20
8	10	12

9	12	15
10	13	16

The results of the study in Table 3 indicate the effect of the aqueous extract of the clove plant on Gram-negative bacteria in the samples used in this study at a concentration of 10% more than a concentration of 5%.

Table 3. Zone of Inhibition Values (MM) of Different Concentrations of Aqueous Extracts of Cloves of Some Gram- Negative Isolates

No. of Sample	Concentration of Aqueous Extract 5%	Concentration of Aqueous Extract 10%
1	5	15
2	13	11
3	15	13
4	5	12
5	7	13
6	10	12
7	12	12
8	9	12
9	12	15
10	14	19

Table 4 shows the effect of the alcoholic extract of the clove plant at a concentration of 10% more than its effect at a concentration of 5% on gram-negative bacteria in the samples under study.

Table 4. Zone of Inhibition Values (MM) of Different Concentrations of Aqueous and Alcoholic Extracts of Cloves of Some Gram- Negative Isolates

No. of Sample	Concentration of Alcoholic Extract 5%	Concentration of Alcoholic Extract 10%
1	17	17
2	15	13
3	15	15
4	14	14
5	12	12
6	15	15
7	12	12
8	10	12
9	12	15
10	15	20

The results of this study indicate that the aqueous and alcoholic extract of the clove plant has an antibacterial effect. The clove plant contains active chemical compounds such as eugenol and alpha-caryophyllene, which may be responsible for this effectiveness in inhibiting bacterial growth [13].

The current study shows that bacteria positive and negative for the Gram stain showed sensitivity to the aqueous and alcoholic extract of the clove plant, due to the ability of the active chemicals in the plant to penetrate the bacterial cell membrane, damage the cell membrane, and destroy the cytoplasm, thus leading to the killing of the cell. This is consistent with other studies presented by the researchers [14], [15], [16], [17].

The results of the current study indicate a direct relationship between the concentration of the plant extract and the diameter of the inhibition zone. It is noted that the higher the concentration of the plant extract, the greater the diameter of the inhibition zone. This is consistent with many studies by many researchers [17], [18], [19], [20].

5. CONCLUSION

The study showed the ability of the aqueous extract of cloves at a concentration of 5% and 10% to inhibit the growth of gram-positive bacteria, as well as the ability of the alcoholic extract to inhibit the growth of the aforementioned bacteria. The results were consistent with the ability of the aqueous and alcoholic extracts at a concentration of 5% and 10% to inhibit the growth of Gram-negative bacteria. The ability to inactivate the aqueous and alcoholic extract was observed at a concentration of 10% more than 5% for both Gram-positive and Gram-negative bacteria. The present study reflected the clove plant to have an actual influence against microbes, also with supplementary laboratory studies of the clove plant, it may be one of the antibiotics that will be used in the future to extravagance microbial infections.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Yusra A. Radeef	✓	✓	✓	✓		✓		✓	✓	✓	✓			

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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