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Study of Antibacterial Activity of Aqueous and Ethanolic Clove Ettract (Syzygium Aromaticum) on Gram Negative Bacteria

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ABSTRACT

The effects of plants aqueous and alcoholic extracts of cloves against some clinical species of microorganisms were studied. These isolates included: Pseudomonas sp., Klebseilla sp., Escherichia coli, and Acinetobacter. The susceptibility of these isolates was tested against plants aqueous and alcoholic extracts by the agar well diffusion method. The results showed that the alcoholic extract of cloves inhibits the growth of Acinetobacter sp., E. coli , Klebseilla sp inhibition zone diameter were (30 , 35 and 20 respectively), While the aqueous extract of cloves inhibits the growth of Acinetobacter sp. , Klebseilla sp. , E. coli inhibition zone diameter were (25,13 and 10 mm respectively). Pseudomonas showed resistance to alcoholic extract while Klebsiella showed resistance to aqueous extract.

1. Introduction

Plants have long been utilized as a medicinal resource throughout several geographical places, serving as a remedy for a diverse range of ailments over millennia. Plants possess the ability to synthesize a diverse range of chemical compounds, enabling them to employ various defense mechanisms against a wide range of diseases. Plants possess a multitude of secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, which have demonstrated antibacterial properties in in vitro experiments. Despite the extensive array of antibiotics currently accessible for the management of bacterial infections, the area of microbial chemotherapy continues to face several challenges. The misuse of these pharmaceuticals might lead to the emergence of resistance towards chemotherapeutic treatments, hence highlighting the need to tackle this issue. The widespread utilization of medications has led to the emergence of antibiotic resistance among microbes, thus reducing the effectiveness of traditional medicinal interventions. Therefore, it is important to examine the advancement of innovative antimicrobial agents in the present context.[1]

Cloves refer to the reproductive structures of the Syzygium aromaticum tree, belonging to the Myrtaceae botanical family. The species under consideration is native to the Maluku Islands, also referred to as the Moluccas, situated in Indonesia. These organisms are mostly utilized in the context of the spice industry. Empirical data suggests that some Syzygium species belonging to the Myrtaceae family exhibit antibacterial and anti-inflammatory activities. It has been asserted that the flower buds of Syzygium aromaticum (L.) Merr. & Perry, commonly known as clove, have been traditionally employed in folk medicine for its diuretic, odontalgic, stomachic, tonic, aromatic condiment, and condiment with carminative and stimulating characteristics. Moreover, it possessed fragrant properties characteristic of a condiment. [2]

Klebsiella pneumoniae is a bacterium characterized by its rod-shaped morphology, Gram-negative cell wall structure, lack of motility, presence of a protective capsule, ability to ferment lactose, and capacity to thrive in both aerobic and anaerobic environments. The specimen has characteristics consistent with a mucoid lactose fermenter when cultured on MacConkey agar. Although they are components of the oral, cutaneous, and gastrointestinal microbiota.[3]

Escherichia coli, referred to as E. coli, is a bacterium belonging to the genus Escherichia. It is characterized by its Gram-negative nature, facultative anaerobic metabolism, rod-shaped morphology, and its presence in the coliform group. E. coli is often found in the lower intestinal tract of warm-blooded species. Escherichia coli is classified as a coliform bacteria due to its characteristic rod-shaped appearance. However, several serotypes of Escherichia coli have the ability to induce serious foodborne illnesses in individuals and are often responsible for instances of product recalls due to food contamination. The vast majority of Escherichia coli strains are benign and pose no significant threat.[4]

Pseudomonas aeruginosa is a prevalent bacterium characterized by its encapsulated, rod-shaped morphology and Gram-negative cell wall composition. This versatile microorganism has the ability to induce infections in a wide range of hosts, including plants, animals, and people. Pseudomonas aeruginosa is widely acknowledged as a bacterium with resistance to several drugs, mostly attributed to its widespread presence, inherent sophisticated mechanisms of antibiotic resistance, and its connection to the development of serious illnesses. Severe diseases such as ventilator-associated pneumonia and other sepsis syndromes are examples of hospital-acquired infections. Pseudomonas aeruginosa is a bacterium that holds considerable medicinal importance.[5]

The genus Acinetobacter is classified as a group of Gram-negative bacteria within the broader class of Gammaproteobacteria. Acinetobacter, a species of microorganisms, is consistently observed in pairs, displaying a characteristic trembling motion, and exhibiting a lack of oxidase enzyme activity when observed under magnification. Bacterial strains classified within the taxonomic genus Acinetobacter are characterized by their Gram-negative cell wall structure, obligate need on oxygen for metabolic processes, and inability to undergo carbohydrate fermentation. These organisms have a deficiency in migratory capabilities, as well as a lack of production of both indole and oxidase, and a near absence of nitrate production. The predominant morphological characteristic observed on nonselective agar is coccobacillary in nature. The user's text is already academic and does not need to be rewritten.[6]

The primary aim of this investigation was to evaluate the antimicrobial efficacy of crude ethanolic and aqueous extracts derived from clove through in vitro experimentation. Additionally, the study sought to enhance the antibacterial properties of clove by incorporating metal ions in different proportions with the crude sample. The target microorganisms for assessment were Escherichia coli, Pseudomonas aeruginosa (a gram-negative bacterium), and Staphylococcus aureus (a gram-positive bacterium) which are commonly associated with food-related issues. The clove extracts were utilized to investigate the susceptibility of three bacterial strains.

2. Material and methods:

2.1. Preparation of clove:

The laboratory received cloves that had through the process of drying and storage, after their acquisition from local retail establishments. The samples were let to undergo natural drying before being individually pulverized into a fine particulate form using a grinder (Model A210, IWATANI, Japan). Subsequently, the pulverized samples were maintained in plastic containers until they were employed in accordance with the guidelines outlined in Table (3.1).

2.2. Ethanol extracts:

A quantity of 50 grams of cloves was placed in sterilized bottles and allowed to soak in 200 milliliters of ethanol for duration of one night, at a temperature of 20 degrees Celsius. Subsequently, the filtrate was subjected to filtration using sterilized Whatman filter paper (No. 2). Subsequently, it was dissolved in a sterile solution of distilled water.

2.3. Aqueous Extracts:

A total of 50 grams of cloves were placed in sterilized bottles and subjected to a soaking process in 200 milliliters of ethanol for a period of one night, at a temperature of 20 degrees Celsius. Following that, the filtrate underwent filtering with sterilized Whatman filter paper (No. 2). Afterwards, it was dissolved in a sterile solution consisting of distilled water.

2.4. Agar well diffusion method

The well diffusion method was employed to determine the antibacterial efficacy. Multiple wells were created in the nutritional agar medium to accommodate the bacteria. Following the inoculation of the plates with 0.1 ml of a bacterial suspension containing 108 colony-forming units per milliliter, a volume of 0.1 ml of plant extract was then administered to each well of the plate.

A decision was made to generate triplicates of every concentration for each distinct microbial species. Following inoculation, the plates were incubated at a temperature of 37 degrees Celsius for a duration of 24 hours. The diameter of each plate's inhibitory zone was measured to estimate its size.

3. Results

In recent years, there has been an increase in scholarly investigation and manufacturing of natural antimicrobials. These substances are employed to impede the proliferation of bacteria responsible for both foodborne illnesses and the spoilage of food products. Cloves have been long utilized in the domains of Ayurveda, Chinese medicine, and Western herbalism, not only for their carminative properties, which aid in relieving flatulence, but also for their ability to enhance hydrochloric acid production in the stomach and promote peristalsis. In contrast to the findings obtained from the water extraction, the ethanolic extraction of clove exhibited significantly elevated levels of inhibitory activity against all four bacterial strains .

Samples of the medicinal plant clove were subjected to a battery of tests in order to ascertain their potential antibacterial properties against four distinct bacterial strains, namely Pseudomonas sp., Klebsiella sp., Acinetobacter sp., and Escherichia coli.

Table (3.1): Antibacterial activity of ethanolic and aqueous extract of clove against food associated gram negative bacteria.

	Inhibition zone (mm)				
	Acinetobacter	***Klebsiella***	***E. coli***	***Pseudomonas***	**Ethanol**
Alcoholic	30	35	20	-	10
Aqueous	25	-	13	10	10

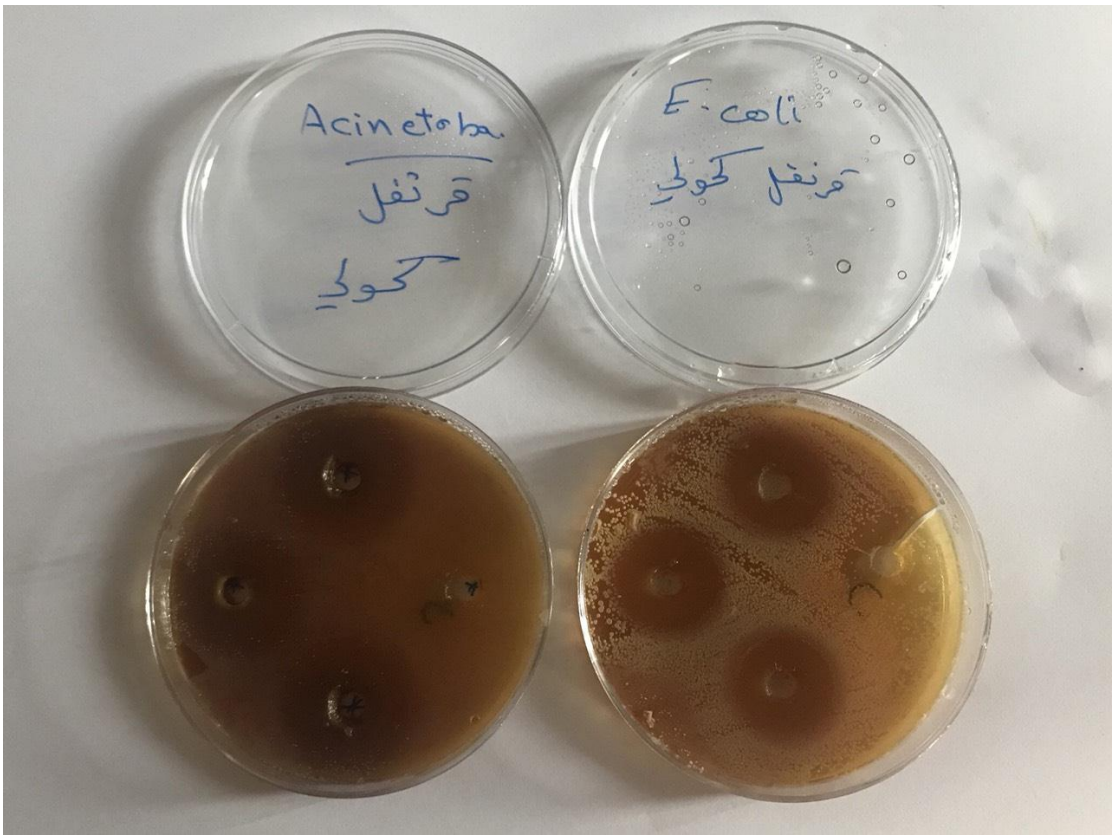

Figure 3.1: Antibacterial activity of ethanolic extract of clove against *Acinetobacter* and *E. coli*.

4. Discussion:

The growth of Acinetobacter sp., E. coli, and Klebsiella sp. was seen to be inhibited by the aqueous extract of cloves, as evidenced by the inhibition zone widths of 30, 35, and 20, respectively, as presented in Table 3.1 and Figure 3.1. The growth of Acinetobacter sp., Klebsiella sp., and E. coli was shown to be inhibited by the alcoholic extract of cloves, resulting in inhibition zone diameters of 25 mm, 13 mm, and 10 mm, respectively. According to the data shown in Table 3.1 and Figure 3.1, it can be observed that Pseudomonas demonstrated resistance to the alcoholic extract, whereas Klebsiella displayed resistance to the aqueous extract.

Several studies have been conducted on a wide range of plant extracts, revealing their ability to exhibit antibacterial properties against diverse microbes. Consequently, further investigation into alternative plant extracts might prove beneficial in exploring their potential as antibiotics for combating various illnesses .

Based on the study conducted by various scholars, it has been observed that the oil and water extracts derived from clove has the ability to effectively eliminate a wide range of bacteria. However, it is well accepted that variations in primary oil components and their corresponding antibacterial properties are impacted by the geographical locations where the plants were grown.

The plant known as clove (Syzygium aromaticum) is cultivated in several regions, including the Spice Islands, Indonesia, Pemba, and Zanzibar. It is worth noting that China was the original producer of this plant. In a manner akin to thyme, this herb is employed for the purpose of seasoning culinary dishes.The numerical values provided are 4 and 6. The antibacterial efficacy of this substance was established via the observation that extracts of its essential oil exhibited bactericidal activity against a wide range of Gram-positive and Gram-negative bacteria, as well as some fungal species.The numerical values provided are 6 and 7. The antibacterial properties of clove are attributed to the presence of eugenol, oleic acids, and lipids in its essential oils.

5. Conclusion

Based on the outcomes of the study and the ensuing discussion, it is possible to reach the following conclusion.

1. The results of the study revealed that the alcoholic extract of cloves had inhibitory effects on the growth of Acinetobacter sp., E. coli, and Klebseilla sp. The sizes of the inhibitory zones exhibited by these pathogens were measured to be 30, 35, and 20, respectively.
2. The aqueous extract derived from cloves exhibits the capacity to inhibit the growth of Acinetobacter sp., Klebsiella sp., and E. coli. The inhibitory zones for the three species were measured to have widths of 25 mm, 13 mm, and 10 mm, respectively.
3. The bacterium Pseudomonas exhibited resistance to an alcoholic extract, but Klebsiella shown resistance to an aqueous extract.

6. Recommendation

Evaluate the antimicrobial activity of crude ethanolic and aqueous extracts of clove against other type of bacteria.

7. References

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