Antibacterial Activity of Clove Extracts on Growth of Some Pathogenic Bacteria

Ghaidaa Neamah Kadhim[1], MADEHA H. HUSSAIN[1], Emad M. Rashed[1] and ALI S. HASSOON[2]

[1]Medical Institute Tech. Mansour, Middle Tech. University, Iraq

[2]Musaib Tech. College, Al-Furat Al-AwsatTech.University, Iraq
Corresponding author e-mail: com.hs.ali@atu.edu.iq ghaidaanehma@mtu.edu.iq

Abstract

The disc diffusion technique was used to determine the antibacterial activity of aqueous and alcoholic extracts of clove against two harmful bacteria *(Staphylococcus aureus and Escherichia coli)* obtained from the Bacteriology laboratory at the medical technical institute-Almansour. The result were showed that the alcoholic extract of clove have more activity than aqueous one in growth inhibition of tested bacteria, and the *Staph. aureus* as a gram positive bacteria was inhibited in their growth more than *E.coli* as a gram negative bacteria. The zone of growth inhibition of *Staph. aureus* in aqueous extract was (14mm) and in alcoholic extract was (18mm) , while in *E.coli* the inhibition zone of growth inhibition was (10mm) in aqueous extract and (14mm) in alcoholics extract comparing with standard antibiotics Gentamicin (20mg/2ml) which shows (24mm) for *Staph. aureus* and (24mm) for *E.coli* .

Keywords: Clove extracts, Disc diffusion method, pathogenic bacteria.

Introduction

Since our ancestors discovered the use of spices in food preservation and in the treatment of clinical diseases, many studies have emerged on the development of antibiotic resistance in bacterial infections, including salmonella (1). Long before the invention of modern medicine, plant-derived products were utilized for medicinal purposes.

The entire population relies on bacterial preparations as medication to suit their needs, and it is believed that around 80% of the world population does so since they are regarded safe and shown to be beneficial against specific disorders. (2).

Cloves *(Syzygium aromatium)* are the dried flower buds of a Myrtaceae tree that are highly aromatic when dried (3). Cloves are used as a carminative to help accelerate peristalsis and boost hydrochloric acid production in the stomach and digestive tract. When it comes to dentistry, it's also useful since clove essential oil may be used as an anadyne in the event of a dental emergency. Aspects of the loves that have anti-mutagenic, anti-inflammatories, antioxidants, antiulcerogenic, antithrombotic, and antiparasitic effects may also be detected (4).

Ferulic acid, beta-caryophyllene, 2-heptanone(5), acetyleugenol, Kaempferol, Eugenol,

isoeugenol, and methyleugenol(6) have all been discovered as components of clove. Phenylpropanoids like carvacrol, thymol, eugenol, and cinnamaldehyde are the major constituents of essential oils (3).

The hunt for novel antibacterial chemicals is an alternate strategy to address pathogenic bacteria resistance to antibiotics, therefore herbal products are increasingly drawing researchers' attention to the development of improved medications to combat resistant bacteria (7,8).

The goal of this investigation was to see if clove aqueous and alcoholic extracts have antibacterial activity against gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria isolated from clinical infections.

Materials and Methods

1) Collection of Plant: -

In this investigation, the clove flower buds employed in the experiment were bought from a Baghdad local market.

2) Bacterial Test Isolates: -

Two clinical bacterial isolates were chosen in this study. The bacteria Were isolated from clinical samples (blood, Wound), and identified as *Staphylloccous aureus* (Gram positive bacteria) and *Escherichia coli* (Gram negative bacteria). The bacteria under investigation were cultivated on nutrient agar plates and incubated at37c° for 24 hours before being stored as stuck cultures in our laboratory (9).

3)Preparation of Clove Extracts: -

a) Preparation of Aqueous Extract

The aqueous extract of clove was made by putting 50g clove in 250 distilled water in a flask and shaking it for two weeks at room temperature, then filtering it through filter paper (Whatmann no. I) to get a clear extract that was maintained in a refrigerator at 4c°.

b) Preparation of Alcoholic Extract

This extract was prepared in the same manner as the aqueous extract, using of methanol 95% instead of distilled water.

4) Diagnosis of active compounds in cloves

On a reversed phase (3 μm) particle size, FLC (Fast Liquid Chromatography) was used to separate the principal chemicals from the secondary compounds (50 x 2.0 mm I.D) C-18DB is a column in the database. The separation was conducted tgerough employing liquid chromatography (Shimadzu 10AV-LC) equipped with a binary delivery pump model (LC-

10A Shimadzu), and the eluted peaks were were observed by utilizing shimadzu SPD 10A vp. The following shows the ideal condition for separation:

Column: FLC (Fast Liquid Chromatographic) column , 3 μm particle size, (50 x 2.0 mm I.D) C-8DB column

Mobile phase :acetonitrile : tetrahydrofuran (THF):,0.1 % asetic acid (6 : 3 : 1, V/V)

detection : UV set at 254 nm

flow rate 1.2 ml/min.

temp: 40 C.

The eluted active compound standard sequence were represented as 25ug/ml for each standard.

Table1: The Retention Time and Active Compound Area

Seq	Subjects	Minutes of retention	Area	Condensation
1	Ferulic acid	3.40	120032	25ug/ml
2	Caffeorylquanic	4.15	134387	25ug/ml
3	Kaempferol	4.67	116653	25ug/ml
4	Eugenol	5.78	96241	25ug/ml
5	Ferulic acid	6.33	97511	25ug/ml

The HPLC separation profile revealed different chromatographic peaks in the studied cloves sample extract. The analysis of the separated substances that comprise the principal observed peaks, as well as the summarization of the collected data for each discovered chromatographic peak, are detailed further down. The peak area of authentic standard and sample peaks were compared under the same optimum separation circumstances, using the equation:

$$\text{Condensation of sample } \mu g/ml = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{conc. of standard} \times \text{dilution Factor}$$

5)Screening of Antibacterial Activity of Clove Extracts: -

The usual disc diffusion technique was employed to test the antibacterial activity of two extracts (10). Sterilized fitter sheets discs (6mm in diameter) were soaked in 5ml of two

extracts for two minutes each and then used for screening. The foundation medium was Mueller-Hinton agar, and the inoculum was prepared with Mueller-Hinton broth. Using a sterile inoculating loop, four to five colonies of the investigated organism were selected and cultured in Mueller-Hinton broth tubes. After 24 hours at 37 c°, the infected tubes were matched with an O.5 McFarland turbidity standard using a nephelometer to determine the turbidity of the sample (11). To inoculate the whole surface of Mueller-Hinton plates, a sterile cotton swab was dipped into bacterial test solution. With the use of sterile for cep, discs of two extracts were put on the surface of inoculation plates. The discs of two extracts were compared to a typical antibiotic disc soaked in Gentamicin (20mg/21ml). After 24 hours of incubation at 37 c° to produce inhibition, the widths of the inhibition zones were determined down to a millimeter accuracy on each plate (mm).

Results

The presented findings displayed that the antibacterial of alcoholic extract was more activity than aqueous extract in growth inhibition of two tested bacteria and Staph-aureas was more sensitive to both extract than *E.coli*.

The result also showed that the diameters of zone inhibition in growth of Staph- aures in aqueous extract was (14mm) and in alcoholic extract was (18mm) while the diameter of zone of inhibition in growth of *E.coli* in aqueous extract was (10mm) and in alcoholic extract was (14mm) .

There result were compare with the activity of Gentamicin (20mg/2ml) as a standard antibiotic that have a diameters of growth inhibition zone of (24mm) in *Staph-aureous* and (22mm) in *E.coli* .

Tables (2) ,(3),(4) and Figure (1) , (2) .

Table2: Concentration of active compound in the aqueous and alcoholic extract of cloves

Phenols	Concentration(%)	
	Aqueous extract	Alcoholic extract
Ferulic acid	7.65	8.82
Caffeorylquanic	11.23	13.05
Kaempferol	9.55	11.89
Eugenol	13.43	15.05
Ferulic acid	10.42	11.92

Table 3 : Clove aqueous extract bactericidal activity in investigated bacteria.

The tested bacteria	Clove extract – Gentamicin	Inhibition Zone (mm)
Staph.aureous	Clove aqueous extract	14
E.coli	Clove aqueous extract	10
Staph.aureous	Gentamicin	24
E.coli	Gentamicin	22

Table 4 : Clove alcoholic extract antibacterial activity in investigated.

The tested bacteria	Clove extract – Gentamicin	Inhibition Zone (mm)
Staph.aureous	Clove alcoholic extract	18
E.coli	Clove alcoholic extract	14
Staph.aureous	Gentamicin	24
E.coli	Gentamicin	22

Figure 1 : Clove alcoholic extract inhibits *Staph. aureus* growth zone.

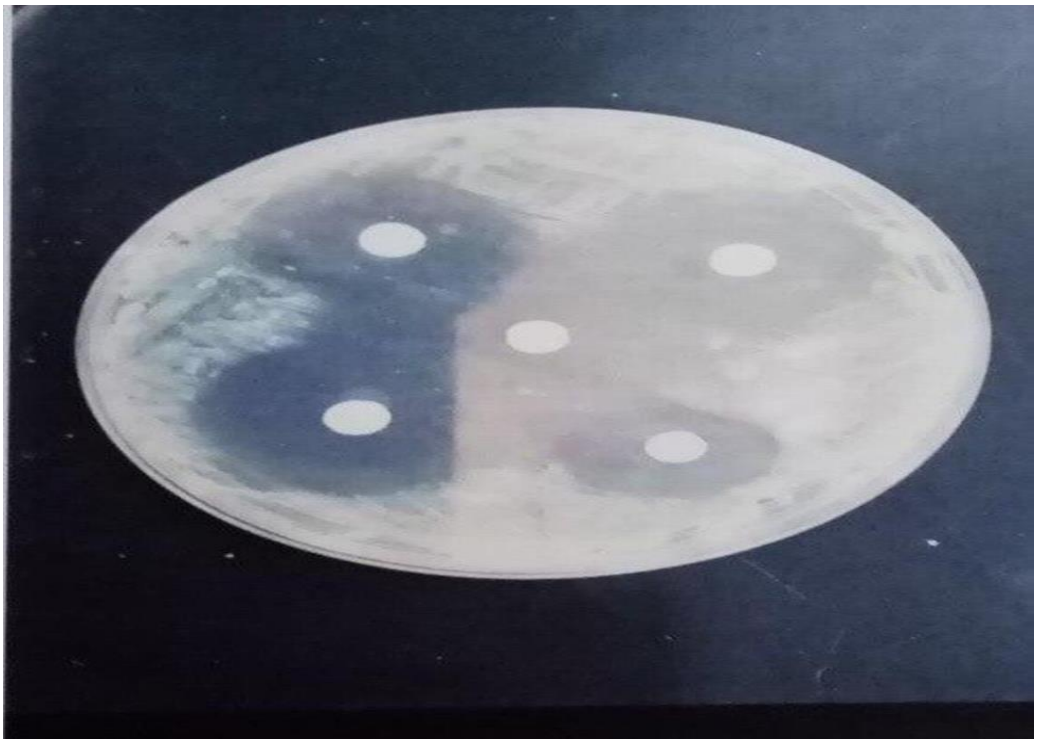

Figure 2: Clove alcoholic extract inhibits *E.coli* growth zone.

Discussion

Clove extracts are known to have antimicrobial properties and are used as taste enhancers in a variety of foods as well as in herbal medicine (12) .The two extracts of clove displayed perfect inhibitory activity in *Staphyloccous aureus* growth and the least on *E-coli* .

The current study's findings are similar to those published by (13) Similarly, clove oil was shown to be active in *S.aureus* and *E.coli* in another investigation (14) and clove extract had an inhibitory effect against *S.aureus* in another study (15).

The antibacterial activity of clove extracts could be associated with Engenol and Tannin content , furthermore the clove extracts have active constituent including: "myricetin , gallic acid , ellagic acid and oleanoic acid possess antibacterial activity" in gram negative bacterial (16).

These components, acting alone or in combination, may display a wide range of antibacterial activity in the microorganisms tested.The extracts with limited or narrowed antibacterial activity .This abservation is in contrast with finding of (17) where extracted materials contained agents that killed the tested bacteria .

The differences in the individual findings may be due to the differences in the methods of extraction and may attribute to the evaporation of the essential active agents in water and alcohol . The alcoholic extract has more activity than aqueous extracts in the growth of tested bacteria , this might be owing to the clove's active ingredients being more soluble in

methanol than in pure water.

The results of our study showed more activity of two extracts against G+ bacteria compared with G- bacteria. This variation in growth inhibition could be explained by differences in the composition and structure of G+ and G- bacteria, as well as the fact that G- bacteria contains an outer membrane which comrises from phospholipid bilayers, that may act as a protective barrier in any active compound in the two extracts (18) (19) .

Any spice's antibacterial effectiveness varies considerably, depending on the kind of spice, the test medium, and the microbe. On the basis of these findings, more chemical and pharmacological investigations into clove extract are necessary. This study's in vitro results show that both clove extracts are potentially powerful antimicrobials and food preservatives. These findings corroborated(20),(21), and (22).

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