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Studies on The Antimicrobial Activities of Two kinds of Clove oil on Some Clinical Pathogenic Bacteria

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Abstract: In the present investigation, we studied the effect of two kinds of Clove oil collected from pharmacies against Gram positive and Gram negative bacteria. The results showed that C. O. 2 had higher inhibitory activities against all tested bacteria than C. O. 1. The highest inhibition zones were for C. O. 2 against *E. coli* and *S. aureus* ssp.2 with diameters 22.33 and 21.67 mm respectively. C. O. 1 was less effective against all tested bacteria. The highest inhibitory activities for C. O. 1 was against *S. aureus* ssp.2 with inhibition zone 14.67 mm.The results of MIC for both of C. O. 1 and C. O. 2 showed that C. O. 2 was more active than C. O. 1 against all tested bacteria. The highest inhibition zone for *S. aureus* ssp.1, *S. aureus* MRSA and *P. aeruginosa* were 23.33, 20 and 24 mm respectively at the concentration 0.18 µml/ml, and the highest inhibition zone for *S. aureus* ssp.2, *E. coli* and *K. pneumonia* were 23, 24.33 and 20.33 mm respectively at the concentration 0.14 µml/ml.The highest inhibition zones caused by C. O. 1 at the concentration 0.18 µml/ml for *E. coli* and *P. aeruginosa* were 20.67 and 22.33 mm respectively. *S. aureus* MRSA was inhibited at the concentration 0.14 µml/ml with a diameter of 14.67 mm. At the concentration 0.12 µml/ml, the inhibition zones for *S. aureus* ssp.1, *S. aureus* ssp. 2 and *K. pneumonia* were 17, 21.33 and 18.33 mm respectively

Keywords: Clove oil, antimicrobial drugs, *Syzygium aromaticum*,

INTRODUCTION

There has been an increasing interest in essential oils during recent years because of the need of new therapies against pathogenic microbes. Bacterial resistance is spreading throughout the world primarily due to excessive use of antibiotics and poor infection control practices in hospitals, making it one of our times biggest issues.

Commercial antimicrobial drugs have been commonly employed as the treatment of infectious diseases for many years, however, in recent years, the indiscriminate use of these antibiotics have developed multiple resistances and side effect. Therefore, more natural antimicrobial substances from plants are desired. A large number of herbs possess antimicrobial activity[1] and some active components of them have become potential source of new anti-infective agents[2].

Essential oils, herbal extracts, are well known for their antimicrobial activity[3]. They are widely used in medicine and food industry for this purpose. Clove (*Syzygium aromaticum*) constitutes one of the major spices. Cloves are dried unopened floral buds of an evergreen tree, *Syzygium aromaticum* belonging to the family *Myrtaceae*[4]. Clove is used as flavoring agent and as spice for scenting, chewing tobacco. It is aromatic, stimulant & carminative, used for dyspepsia and gastric irritations[5].

The essential oils from clove (*Syzygium aromaticum*) is natural substances that are not harmful when consumed in medicine and food products. There have been some reports about clove essential oils' activity that inhibited the growth of bacteria and fungi. Antimicrobial properties of clove essential oil was tested and showed inhibitory activity to *Listeria monocytogenes, Campylobacter jejuni, Salmonella enteritidis, Bacillus cereus, Escherichia coli* and *Staphylococcus aureus*[6].

Tony *et al*.[7], studied the activities of the volatile oils of *Syzygium aromaticum, Thymus serpyllum, Lavandula angustifolia* and *Lavandula x intermedia* against five organisms; methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), *Pseudomonas aeruginosa, Streptococcus pyogenes* and *Candida albicans* and The results showed considerable variability in the size of zone of inhibition depending which oil was used and no essential oil was observed to be the "best" against all organisms.

Ali *et al*.[8] studied the effect of the clove oil's MIC against some tested superinfectant organisms like *Cadida albicans* which was demonstrated at a concentration of (24µg/ml). The MIC for *Pseudomonas aeruginosa,* and *Staphylococcus aureus* was (24µg/ml) but for *Escherichia coli* was (18µg/ml).

The aim of this study was to determine the antimicrobial activities of two different sources of Clove oils against six different microorganisms: *Klebsiella pneumonia, Staphylococcus aureus* ssp.1, *Staphylococcus aureus* ssp. 2, *Staphylococcus aureus* MRSA, *Escherichia coli* and *Pseudomonas aeruginosa.*

MATERIAL AND METHODS

Materials:

Tested oils: The two kind's Clove oil which were used in the present study were chosen from some famous pharmacies in Jeddah city and they were manufactured by different companies but were similar in their uses for the toothache. They named (C. O. 1) which is from Al-Hayat Factory for Medical Products and (C. O. 2) which is made from Gulf Care Factory for Healthcare & Cosmetics Products.

Tested bacteria: All Tested pathogenic bacteria were isolated and classified in the Microbiology lab in KFAFH in K. S. A.

• Gram negative bacteria: some pathogenic strains were used for screening and they were *Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa*.

• Gram positive bacteria: The most pathogenic bacteria which almost found in the patients were *Staphylococcus aureus* ssp.1, *Staphylococcus aureus* ssp.2 and *Staphylococcus aureus* MRSA.

Culture Media:• McConky agar and Cled agar were used to study the antibacterial activities of the tested Clove oils against Gram negative bacteria *E. coli, K. pneumonia* and *P. aeruginosa*.

• Chocolate agar and Sheep blood agar medium were used for screening the antibacterial activities against *Staphylococcus aureus* species.

Methods:

Screening the Antimicrobial Activities of Clove oils: The antimicrobial activities of the tested kinds of Clove oils were studied *in vitro* using the whole plate diffusion method which called well diffusion method [9]. Mac Conky agar and Cled agar were used for the rest of the Gram negative isolates, Chocolate agar and Sheep Blood agar were used for *Staphylococcus aureus* isolates. The solid agar was punched with 5 mm diameter wells. The inoculums were spread on the surface of each plate using sterile swabs and then filled with 0.1 ml tested clove oils. The plates were then incubated at 37 °C for 24h. After incubation, the inhibition zone of each plate was measured.

Determination of Minimum Inhibitory Concentration (MIC) of Clove oils: The tested clove oil samples that showed antibacterial activities were tested to determine the minimum inhibitory concentration (MIC) for each bacterial sample. The MIC of the tested clove oils against the individual test organisms were determined by well diffusion method. Ten concentrations of the tested clove oils for each sample were prepared by using Tween 80 to achieve a concentration range of approximately (0.18, 0.16, 0.14, 0.12, 0.1, 0.08, 0.06, 0.04, 0.02 µml of oil/ml Tween 80) (v/v). The solid agar was punched with 5 mm diameter wells. The inoculums were spread on the surface of each plate using sterile swabs and then filled with 0.2 ml tested clove oils concentrations. The plates were then incubated at 37° C for 24h and after which the diameter of inhibition zone of each plate was measured [10].

Statistical analysis: Mean diameter of zone of inhibition and standard deviations were calculated by using SPSS. T-Test at Significant level 95%.

RESULTS

In the present study, two different kinds of Clove oil that used for toothache were tested against seven pathogenic bacteria. The data in table (1) and fig. (1) Showed that Blood agar was found to be better than Chocolate agar and Macconky agar was also found to be better than Cled agar for studying the antibacterial activities of C. O. 1 and C. O. 2 for all tested pathogenic bacteria. It was found that the C. O. 2 was potentially active against all tested bacteria in all media than C. O. 1. Fig. (2) Showed that *E. coli* was found to be the most highly sensitive to C. O. 2 with 25.33 mm inhibition size on Macconky agar. Fig (3) showed that the inhibition zone for *S. aureus* ssp. 2 was 21.67 mm with C. O. 2. Figs. (4 and 5) showed the highest inhibition zones for *K. pneumonia* and *P. aeruginosa* with diameter 13.67 mm and 19.33 mm respectively.

Another result had shown in that assay. The plastic Petri dishes which had used in this assay had analyzed because of the Clove oil (C. O. 1) after 24 hours in the incubator at 37 °C. Pictures (6A, 6B,

6C, 6D, 6E and 6F) showed these results. But the petri dishes which contained the Clove oil (C. O. 2) didn't analyze.

Table 1: Antibacterial activities of C. O. 1 and C. O. 2 against tested pathogenic bacteria.

Tested microbes	Growth media	C. O. 1	C. O. 2
S. aureus ssp.1	Blood agar	6.67 ± 0.01	14.67 ± 0.02
	Chocolate agar	5.67 ± 0.04	13.33 ± 0.08
S. aureus MRSA	Blood agar	11.33 ± 0.07	15.33 ± 0.04
	Chocolate agar	13.33± 0.09	14 ± 0.03
S. aureus ssp.2	Blood agar	14.67 ± 0.01	21.67 ± 0.05
	Chocolate agar	14.33 ± 0.07	20.33 ± 0.04
E. coli	Macconky agar	6 ± 0.03	25.33 ± 0.05
	Cled agar	7.67 ± 0.03	22.33 ± 0.01
K. pneumonia	Macconky agar	13.67 ± 0.02	13.67 ± 0.09
	Cled agar	13.67 ± 0.04	13 ± 0.03
P. aeruginosa	Macconky agar	6.67 ± 0.02	19.33 ± 0.05
	Cled agar	6.33 ± 0.07	± 0.04

***Significant level 95%**

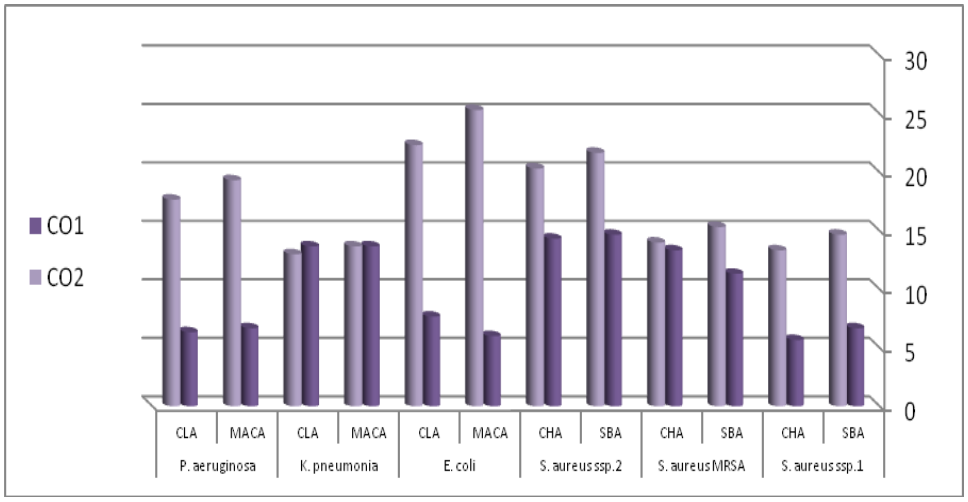

Fig. (1): Antibacterial activities of C. O. 1 and C. O. 2 against tested pathogenic bacteria.

Fig(2): Antibacterial activity of C. O. 2 against *E. coli*

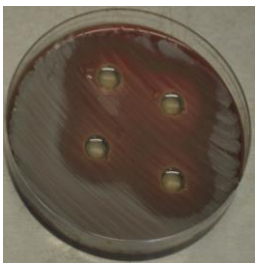

Fig. (3): Antibacterial activity of C. O. 2 against *S. aureus* ssp.2

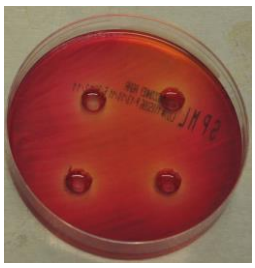

Fig. (4): Antibacterial activity of C. O. 2 against*K. pneumonia*

Fig. (5): Antibacterial activity of C. O. 2 against *P. aeruginosa*

Fig. (6A)

Fig. (6B)

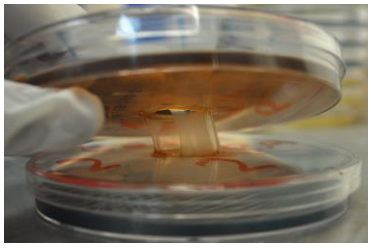

Fig. (6C)

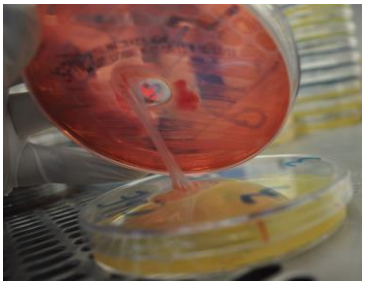

Fig. (6D)

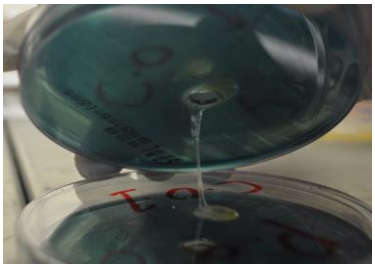

Fig. (6E)

Fig. (6F)

Figs. (6Ato 6F) : The plastic analyzes for Petri dishes with C. O. 1

The MIC values ranged from 0.18 to 0.02 µml/ml by using Tween 80 for making the different concentrations of the C. O. 1 and C. O. 2. The studies of *S. aureus* ssp.1 in table (2) and fig. (7A) showed that all the concentrations of C. O. 2 was better in the effect than all the concentrations of C. O. 1. The best activity treatment with wide inhibition zone was 23.33 mm in the concentration 0.18 µml/ml **(fig. 8A).** The results of *S. aureus* MRSA in table (2) and fig. (7B) showed that C. O. 2 had more inhibitory activities than C. O. 1 in all concentrations. The inhibition zones were from 20 mm to 15.33 mm at the concentrations from 0.18 µml/ml to 0.08 µml/ml. But C. O. 1 hadn't high activities against *S. aureus* MRSA. The biggest inhibition zone for C. O. 1 was 14.67 mm at 0.1 µml/ml (fig. 8B). and the less inhibition zone was 6.67 mm in the concentration 0.02 µml/ml.

C. O. 2 was also having high activities against *S. aureus* ssp.2 in all the tested Concentrations. The results in **table (2)** and **Fig. (7C)** showed that the highest effect of C. O. 2 was in the concentration 0.14 µml/ml and the inhibition zone size was 22.33 mm (fig. 8C). C. O. 1 was less effective against *S. aureus* ssp.2 in all concentrations than C. O. 2. The inhibition zones sizes were between 21.33 mm and 6.67 mm. in the concentration 0.02 µml/ml, C. O. 1 hadn't any activities against *S. aureus* ssp.2.

Table 2: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against the tested gram positive bacteria.

Con.	Gram Positive Bacteria					
	s. aureus ssp.1		*S. aureus* MRSA		*S. aureus* ssp.1	
	C. O. 1	C. O. 2	C. O. 1	C. O. 2	C. O. 1	C. O. 2
0.18	12.67±0.03	23.33±0.06	9.33± 0.09	20± 0.05	12± 0.02	20.33± 0.03
0.16	14.33± 0.05	22.33± 0.07	10.67± 0.03	19.33± 0.02	15± 0.02	21.67± 0.01
0.14	15.67± 0.08	21.33± 0.08	11.67± 0.07	18.33± 0.01	19± 0.06	23± 0.08
0.12	17± 0.04	19.67± 0.04	13.33± 0.02	17.67± 0.07	21.33± 0.03	22.33± 0.02
0.1	14.67± 0.06	18.67± 0.04	14.67± 0.06	16.33± 0.08	17.67± 0.09	20.67± 0.05
0.08	13.33± 0.02	17± 0.09	13.33± 0.02	15.33± 0.02	14.67± 0.06	19± 0.04
0.06	13± 0.03	15.67± 0.03	10.67± 0.07	14.67± 0.05	8± 0.01	17.67± 0.05
0.04	12.33± 0.06	13.33± 0.01	8.67± 0.02	13± 0.06	6.67± 0.04	16.33± 0.03
0.02	11± 0.07	11± 0.01	6.67± 0.04	12.33± 0.08	0± 0.00	14.33± 0.01
0	0± 0.00	0± 0.00	0± 0.00	0± 0.00	0± 0.00	0± 0.00

***Significant level 95%**

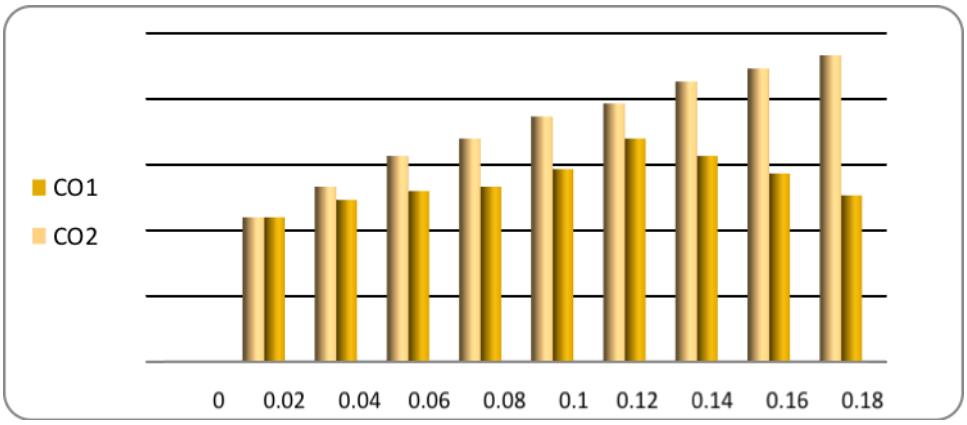

Fig. 7A: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against *S. aureus* ssp.1.

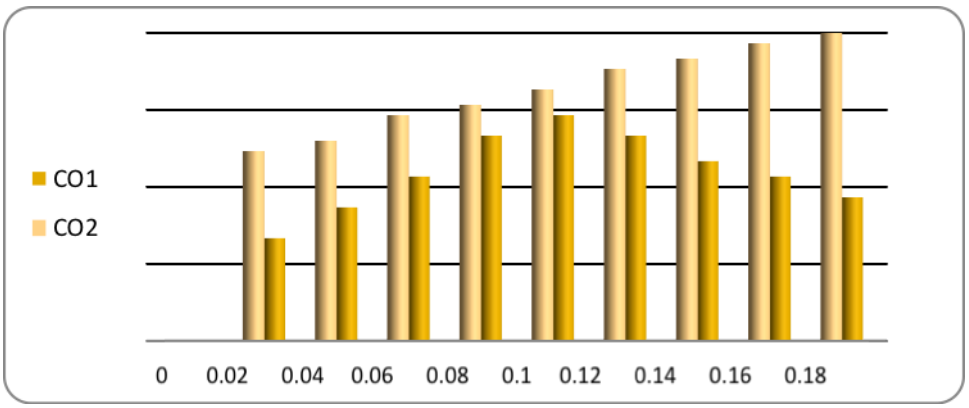

Fig. 7B: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against *S. aureus* MRSA.

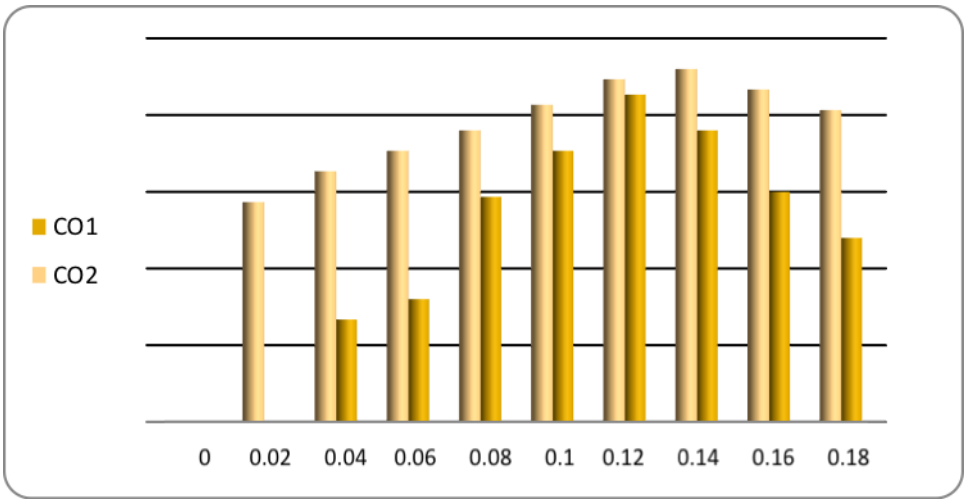

Fig. 7C: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against *S. aureus* ssp.2.

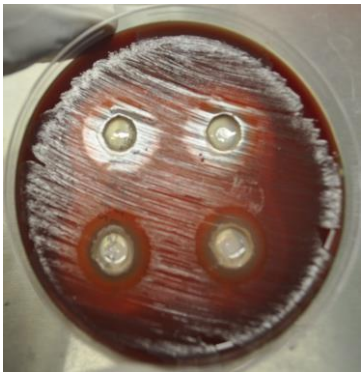	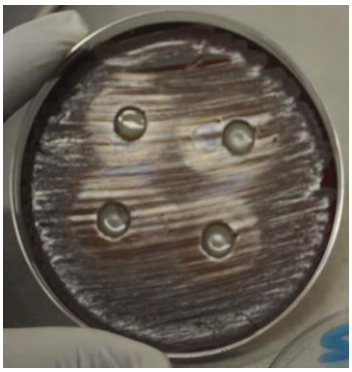	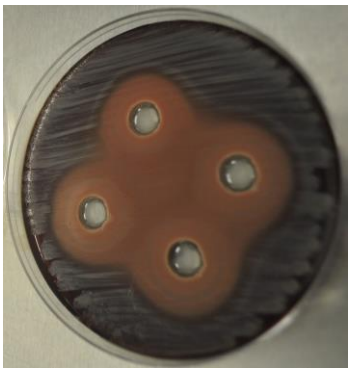
Fig. (8A): showed the antibacterial activity of C. O. 2 against *S. aureus* ssp.1	**Fig. (8B):** showed the antibacterial activity of C. O. 2 against *S. aureus* MRSA	**Fig. (8C):** showed the antibacterial activity of C. O. 2 against *S. aureus* ssp.2

The studies of the MIC of C. O. 1 and C. O. 2 against Gram Negative bacteria isolates showed that C. O. 2 had higher inhibitory activities than C. O. 1. The results in table (3) and fig. (9A) showed that *E. coli* was sensitive for C. O. 1 and C. O. 2 with nearly the same results. The highest inhibition zones were 24.33 mm at the concentration 0.14 µml/ml (fig. 10A) and 22 mm at the concentration 0.12 µml/ml. At the concentration 0.06 µml/ml, the inhibition zone for *E. coli* was 13.67 mm at C. O. 1 and C. O. 2.

C. O. 1 had high inhibitory activities in almost concentrations against *K. pneumonia* and *P. aeruginosa*. Table (3) and fig. (9B) showed that the inhibition zones of C. O. 1 against K. *pneumonia* were between (13.67 and 18.33 mm). Table (3) and fig. (9C) showed that C. O. 1 inhibited P. *aeruginosa* with inhibition zones between (22 and 12.33 mm). But C. O. 2 was less effective than C.

O. 1 against both of *K. pneumonia* and *P. aeruginosa*. The inhibition zones were between 17.33 and 8.33 mm in all concentrations expect 0.14 µml/ml which had the highest inhibition zone 20.33 mm against K. *pneumonia* fig. (10B). C. O. 2 had the highest inhibitory activities against P. *aeruginosa* in the concentrations 0.18 and 0.16 µml/ml with inhibition zones 24 mm and 22.67 mm respectively **(fig. 10C).**

Table 3: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against the tested gram Negative bacteria.

Con.	Gram Negative Bacteria					
	E. Coli		*K. pneumonia*		*P. aeruginosa*	
	C. O. 1-T	C. O. 2-T	C. O. 1-T	C. O. 2-T	C. O. 1-T	C. O. 2-T
0.18	20.67± 0.02	18± 0.06	15.33±0.06	14.67± 0.04	22.33±0.06	24± 0.08
0.16	19.33± 0.04	20.5± 0.03	16.67±0.09	17± 0.05	21.33±0.03	22.67±0.01
0.14	17.67± 0.08	24.33±0.05	17.67±0.04	20.33± 0.07	20.67±0.05	20.33±0.07
0.12	16.33± 0.07	22± 0.03	18.33±0.04	17.33± 0.08	19.33±0.02	18.33±0.02
0.1	15± 0.01	19± 0.03	17.33±0.05	15± 0.03	17.67±0.04	15± 0.03
0.08	14.33± 0.05	15.67±0.04	16.33±0.02	11.67± 0.01	16± 0.01	13± 0.01
0.06	13.67± 0.02	13.67±0.07	14.33±0.06	10.33± 0.02	14.67±0.08	10.33±0.01
0.04	12± 0.01	11.33± 0.07	13.67±0.02	8.33± 0.02	13.33±0.06	9± 0.06
0.02	11.33± 0.01	9.33± 0.09	13± 0.03	7± 0.08	12.33±0.04	7.67± 0.02
0	0± 0.00	0± 0.00	0± 0.00	0± 0.00	0± 0.00	0± 0.00

***Significant level 95%**

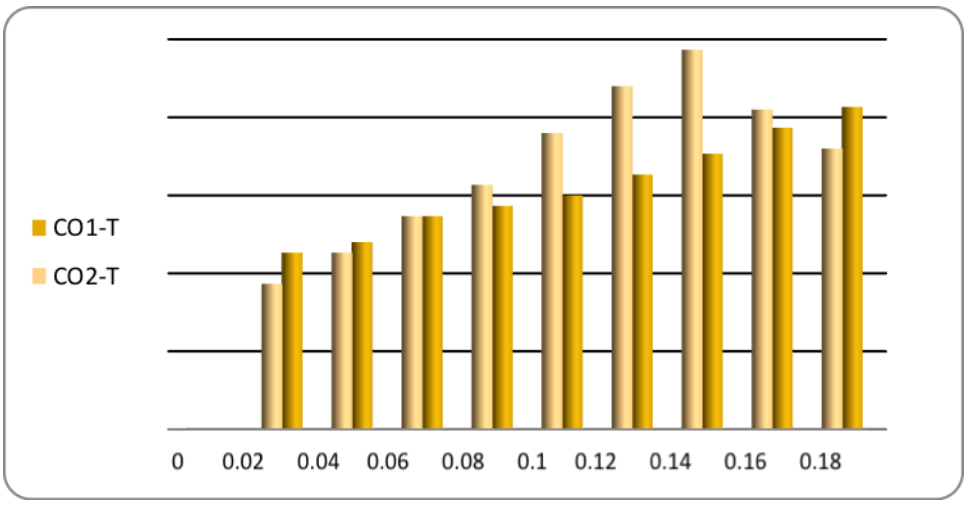

Fig. 9A: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against *E. coli*.

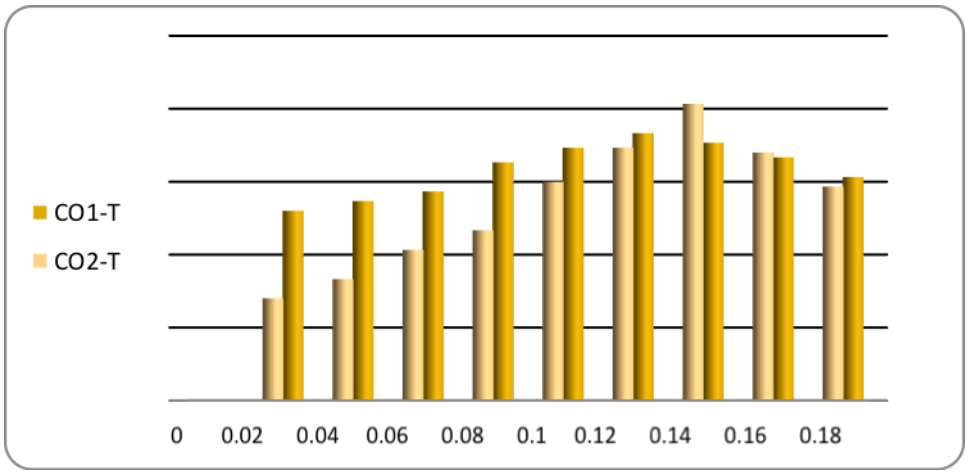

Fig. 9B: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against *K. pneumonia*

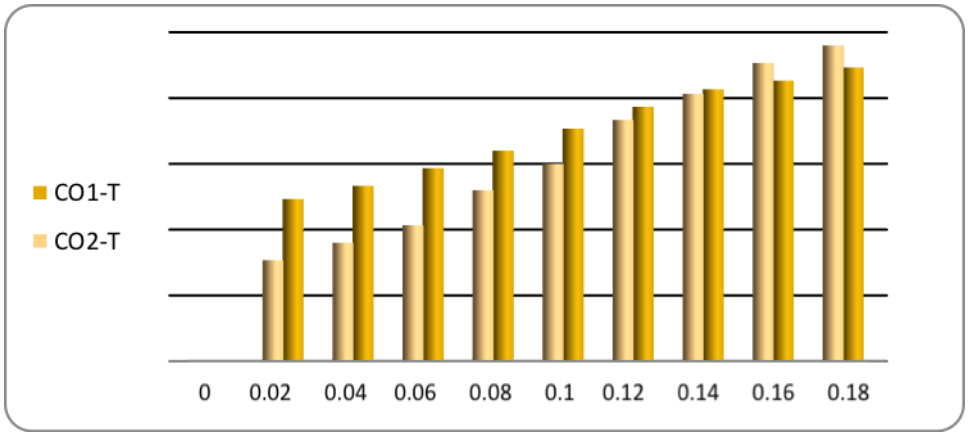

Fig. 9C: Minimum inhibitory concentration (MIC) of Clove oils (C. O. 1) and (C. O. 2) against *P. aeruginosa*

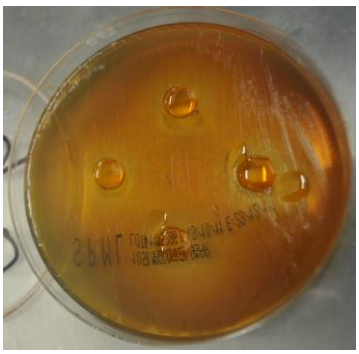

Fig. (10A): showed the antibacterial activity of C. O. 2 against *E. coli*

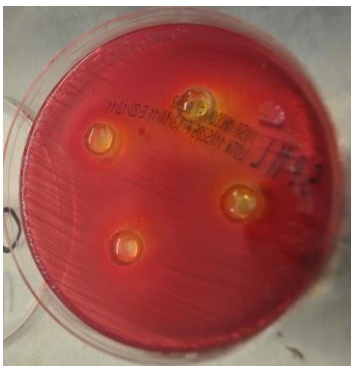

Fig. (10B): showed the antibacterial activity of C. O. 2 against *K. pneumonia*

Fig. (10C): showed the antibacterial activity of C. O. 2 against *P. aeruginosa*

The plastic analyzed again in all Petri dishes in the Concentrations between (0.18 µml/ml and 0.1 µml/ml) for C. O. 1. after 24 hours in the incubator at 37 °C. figs. (11A and 11B) showed these results. But the Petri dishes which contained the Clove oil (C. O. 2) didn't analyze at all.

Fig. (11A)

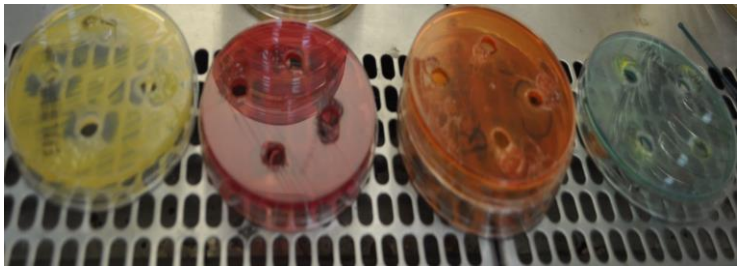

Fig.. (11B)

Figs. (15 A and B): shown the plastic analyze in Petri dishes with C. O. 1 with different concentrations

Discussion

Several studies have reported that Clove oils can inhibit the growth of bacteria in culture media such as **Saeed** & **Tariq**[11], who reported that Clove essential oil of clove exhibited maximum activity against Gram –ve bacilli viz., *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Salmonella typhi*, *Shigella dysentriae* and *Vibrio cholera*. Similarly,[12] found that Clove oil had inhibitory activities against Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *B. megaterium*, *B. polymyxa*, *B. sphaericus*, *Staphylococcus aureus* and *Escherichia coli*).

The inhibitory activity of clove is due to the presence of several constituents, mainly phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid[13] acetyl- eugenol, alpha-humulene, methyl salicylate, iso-eugenol, methyl-eugenol[14], eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone and the main constituents of essential oil are phenyl-propanoides such as carvacrol, thymol, eugenol and cinnamaldehyde[15] . Eugenol (2 methoxy-4 allyl-phenol) [16]. High tannin content (10-19%) in clove also provides additional antimicrobial activity[17].

The modes of action by which microorganisms are inhibited by essential oil and their chemical compounds seem to involve different mechanisms[18]. It has been hypothesized that the inhibition may be due to phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents[19] and/or impairment of bacterial enzymes systems[20].

The components with phenolic structure such as eugenol are highly active against the tested bacteria. Clove oil has 79.2% eugenol [21]. Members of this class are known to be either bactericidal or bacteriostatic agents, depending upon the concentration used[22,23] . These compounds were strongly active despite their relatively low capacity to dissolve in water, which is in agreement with published data[24].

RECOMMENDATIONS

It may be suggested from our results that the Clove oil can be used as a potential source of natural antimicrobial compounds. So we need to make further analysis of Clove oil could be done to isolate

and identify the antimicrobial agents present in the spice and to determine their minimal inhibitory concentrations. Also we must determine those compounds in the Clove oil (C. O. 1) which cause the plastic analyze in Petri dishes and send warning to that company which made the Clove oil (C. O. 1) to review that technical error in the manufacture of the Clove oil.

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