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### Antioxidants and Antibacterial Activities of Bioactive Compounds of Clove (*Syzygium aromaticum*) and Thyme (*Tymus vulgaris*) Extracts

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#### ABSTRACT

Recently, there is an increasing attention in finding antioxidant phytochemicals natural to avoid the effects of synthetic antioxidants and antimicrobial compounds. In this study, two herbs namely clove buds (*Syzygium aromaticum*) and thyme leaves (*Tymus vulgaris*), were used to extract the bioactive compounds. The chemical composition of clove buds and thyme leaves was determined. Ethanolic and aqueous extractions were carried out to obtain biological active compounds. Ethanolic clove-powdered buds extracts had the highest total phenolic compounds (TPC) (372.21 mg GAE/g extract) while, essential oil extract of thyme-powdered leaves was (158.83 mg GAE/g extract). Total flavonoids compounds (TFC) of ethanolic clove-powdered buds extract was (177.15 mg QE/g extract) but aqueous extract of thyme-whole leaves was (126.50 mg QE/g extract). The reducing power of thyme-powdered leaves was (1.022) comparable with vitamin C (8.911) meanwhile, clove-powdered buds extract was (1.031). Free radical scavenging capacity of clove-whole buds was (IC<sub>50</sub>; 2.75 µg/mL) and thyme-whole leaves extracts had (IC<sub>50</sub>; 2.86 µg/mL) comparable with ascorbic acid (IC<sub>50</sub>; 6.75 µg/mL). The antibacterial of all used herbs extracts against *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*. Were studied. On *Escherichia coli*, thyme-powdered leaves essential oil had (19.75mm inhibition zone) while, clove-whole buds ethanolic extract was (14.5 mm inhibition zone). Thyme-whole leaves essential oil was (24.5 mm inhibition zone) against *Staphylococcus aureus*, but clove-whole buds essential oil extract was (12.25 mm inhibition zone). Aqueous extract obtained from thyme-powdered leaves was (14.5mm inhibition zone) While, clove-powdered buds ethanolic extract was (13.5 mm inhibition zone) against *Bacillus cereus*.

**Keywords:** clove buds thyme leaves antioxidant antimicrobial



#### INTRODUCTION

Free radicals are created when cells use oxygen to produce energy. These free radicals are commonly reactive oxygen species (ROS) that produce from the cellular redox process. At little or moderate concentrations, ROS exert useful effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a harmful development that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy *et al.*, 2008). A free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence (Phaniendra *et al.* 2015). Oxidative stress occurs when the balance among reactive oxygen species (ROS) formation and detoxification favors an increase in ROS levels, leading to disturbed cellular function (Adwas *et al.* 2019) that plays a main part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, cataract, rheumatoid arthritis, aging, cardiovascular and neurodegenerative diseases (Willcox *et al.*, 2004 and Pham-Huy *et al.*, 2008). Thyme contains several flavonoids, phenolic antioxidants like zeaxanthin, lutein, pigenin, naringenin, luteolin and thymonin. Thyme herb has one of the highest antioxidant levels amongst herbs. It is chock-full with minerals and vitamins that are essential for optimum health. Its leaves are one of the richest sources of potassium, iron, calcium, manganese, selenium and magnesium Sharangi, *et al.* (2013)

The body of human has several mechanisms to counteract oxidative stress by creating antioxidants, which are naturally produced either in situ, or externally supplied through foods. These antioxidants act as free radical scavengers by stopping and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases (Pham-Huy *et al.*, 2008). In recent years, there is an increasing interest in finding antioxidant compounds, because they can inhibit the propagation of free radical reactions, defend the human body from diseases (Terao and Piskula, 1997).

Clove (*Syzygium aromaticum*) is a nutrient dense food rich in useful phytochemicals and aroma. Cloves have many therapeutic uses: antioxidant, anti-inflammatory and antifungal (Mashkor, 2015). The essential oil extract from the buds of clove is generally used and well known for its medicinal properties (Chaieb *et al.* 2007).

Thyme (*Tymus vulgaris*) and its extracts have been used in traditional medicine for the cure of some respiratory diseases like asthma and bronchitis and for the cure of other pathologies thanks to several properties such as antiseptic, antitussive antimicrobial, antispasmodic, antifungal, antioxidative, and antiviral (Ocaña and Reglero 2012.). Thyme essential oil constitutes raw material in cosmetics and perfumery due to a special and characteristic aroma (Dauqan and Abdullah 2017). Therefore, this study was carried out to evaluate the water and alcoholic extracts of two herbs

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(*Syzygium aromaticum*) and (*Tymus vulgaris*) for determined chemical composition, antioxidant and antibacterial activity.

## MATERIALS AND METHODS

### 1 Plant materials.

Shade-dried clove buds (*Syzygium aromaticum*) and dried thyme leaves (*Tymus vulgaris*) were obtained from Harraz Company, Nasr City, Egypt. These samples manually crushed, filled in polyethylene bags and kept at 3-5°C until use.

### 2 Determination of proximate chemical composition;

Moisture, crude protein, crude fat and total ash contents of dried thyme leaves and clove were determined according to the standard analytical methods of A.O.A.C. (1995). Total carbohydrates was calculated by difference, as follows:

$$[\text{Total carbohydrates \%} = 100 - (\text{moisture} + \text{protein} + \text{intramuscular-fat} + \text{ash})].$$

### 3 Extraction processes:-

#### Ethanolic and aqueous extracts of herbs:

The clove buds whole (120g) were mixed with ethyl alcohol 95% (2.0 L) in 2.5 L flask. The mixture was shaken several times and kept for tow day in the lab shade at room temperature. Another (120g) were mixed with distilled water (2.0 L) instead of ethyl alcohol in the second flask, shaken and kept at room temperature. Thereafter, they were filtered rapidly taking precautions against the loss of the solvent. Another 120 g of clove buds are ground into a powder using an electric blender. The powder sample were mixed with ethyl alcohol 95% (2.0 L) in 2.5 L flask. The mixture was shaken several times and kept for tow day in the lab shade at room temperature. Another (120g) were mixed with distilled water (2.0 L) instead of ethyl alcohol in the second flask. Thereafter, they were filtered rapidly taking precautions against the loss of the solvent. The ethanolic and water extracts of clove buds were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (60°C). The extracts were stored in a refrigerator at 4°C until further use (Harborne, 1998).

The thyme dried leaves whole and powder (120g) were mixed with ethyl alcohol 95% or water (2.0 L) in 2.5 L flask. The mixture was shaken and kept for tow day at room temperature. Thereafter, they were filtered rapidly taking precautions against the loss of the solvent. The ethanolic and the water extracts of thyme leaves and powder were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (60oC). The extracts were stored in a refrigerator at 4oC until further use (Harborne, 1998).

Where EE= Ethanolic Extract Aq= Aqueous Extract  
EOE= Essential Oil Extract

#### Extraction of essential oils.

Extraction of clove and thyme essential oils were conducted by using steam distillation method. Each origin of dried powdered sample weighed for 100 g and then placed on 2000 mL distillation flask. Another 100 g whole dried sample placed on 2000 mL distillation flask, 1.5 L of distilled water as a solvent were added to each flask. Steam distillation process done for 4 hours when no oil dropped out for volatile distillate. After the water-oil separation is carried out, the oil is dried by passing it on anhydrous sodium sulfate Dry pure oils were weighed to calculate the yield of clove and thyme

oil. The oils were stored in a refrigerator with temperature 4° C in a sealed vial prior to analysis (Romeilah, 2009).

#### Determination of total phenolic compounds (TPC)

Total phenolics compounds (TPC) were determined using Folin-Ciocalteu method according to Li *et al.* (2007). The absorbance was measured at 750 nm. Total phenolic compounds were expressed as milligram gallic acid equivalent (GAE)/g extract.

#### Determination of total flavonoids compounds

Used aluminum chloride colorimetric method for quantitative flavonoids determination according to the method described by Chang *et al.*, (2002). The absorbance was measured at 415 nm. Total flavonoids content was expressed as mg of quercetin equivalent (QE) / g extract.

#### Determination of antioxidant activity:

##### Estimation of reducing power.

Reducing power assay was determined as described in the method of Ferreira *et al.* (2007). Ascorbic acid was used as reference standard. Absorbance was measured at 700 nm against the corresponding blank solution.

##### Determination of radical scavenging activity (DPPH %):

2, 2 -diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity was determined according to the procedure described by Martins *et al.* (2008). Sample solution was diluted with DMSO and in each reaction mixture, the solution was mixed with 2.0 ml of 100 µM DPPH. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. in dark. Decolorization of DPPH was determined by measuring the absorbance at 517 nm.

$$\text{DPPH scavenging activity\%} = (A_0 - A_1/A_0) \times 100$$

A<sub>0</sub>: Absorbance without extract (blank).

A<sub>1</sub>: is the absorbance in the presence of the extract or standard sample.

##### Screening of the antibacterial activity of herbal extracts

Antibacterial activity of herbs extracts was performed using well agar diffusion method according to Boyanova *et al.* (2005). Petri plates containing 15-20 ml of nutrient agar medium were inoculated with a culture of the studied bacterial strains for a 24 h. Wells (6 mm diameter) were cut into the agar using sterile cork borer. Using sterilized dropping pipettes, extracts were carefully added into the wells. Serial concentrations of all tested extracts were achieved (w/v) in plates containing nutrient agar medium, as follow: 10, 20, 30 and 40 mg/ml concentrations in dimethylsulfoxide (DMSO) were filled to the holes using a pipette. DMSO without extracts was used as a control. After inoculation procedures, extracts and control plates were then incubated at 35°C. The plates were evaluated for the presence or absence of visible growth of each bacterial species *Escherichia coli*, (*E. coli*) *Staphylococcus aureus* (Staph.) and *bacillus cereus* (Bacillus) on the agar plate after 24 h of incubation. The absence of colonies on tested plates (zone inhibition) were measured and expressed as (millimeter).

## RESULTS AND DISCUSSION

### 1. Chemical composition of herbs used.

Moisture, crude protein, fat, ash, and total carbohydrates were determined in dried clove buds and dried thyme leaves, and the results are presented in Table (1). From the obtained results, it could be noticed that dried thyme leaves recorded the higher contents of ash (7.86%), crude protein (9.67 %), and total carbohydrates (61.40%) than those of dried clove buds. While dried clove buds had a higher

contents of moisture (12.29%) and fats (14.36%) than those of dried thyme leaves. These results are in agreement with those of Sulieman *et al.* (2007) who found that dried clove buds contain ash (5.2%), fat (12.1 %), and total carbohydrates (51.1%).

**Table 1. Proximate chemical composition of clove buds and thyme leaves powders.**

Herbs	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %
clove buds	12.29±	6.07 ±	14.36±	6.79±	60.49±
powders	0.34	0.16	0.17	0.14	0.22
thyme leaves	8.59±	9.67 ±	12.46±	7.86±	61.40±
powders	0.68	0.23	0.47	0.19	0.22

**2 Total phenolic and flavonoid compounds of clove and thyme different extracts**

In this study, six different extracts were used from each herbs. A wide range of extracts holds a better chance for the extraction and isolation of biologically active compounds for general screening of bioactivity.

In thyme extracts, essential oil of dried thyme-powdered leaves presented the highest amount of total phenolic compounds TPC (158.83 mg GAE/g extract), and the lowest was observed in the aqueous extract obtained from dried thyme-whole leaves (46.50 mg GAE/g extract). While, aqueous extract obtained from dried thyme-whole leaves presented the highest amount of total flavonoid content (TFC) (126.50 mg QE/g extract) and the lowest was observed in the ethanolic extract obtained from thyme-whole leaves (53.53 mg QE/g extract). These results are agreement with Köksal *et al.* (2016)

In clove extracts ethanolic extract obtained from clove-powdered buds presented the highest amount of total phenolic compounds TPC (372.21 mg GAE/g extract), and the lowest was observed in the aqueous extract obtained from clove-powdered buds (158.56 mg GAE/g extract). While ethanolic extract obtained from clove-powdered buds presented the highest amount of total flavonoid content (TFC) (177.15 mg QE/g extract) and the lowest was observed in the aqueous extract obtained from clove- powdered buds (39.46 mg QE/g extract). These results are in agreement with those El-Maati *et al.* (2016) who reported that ethanol and water were the best solvents for extracting phenolic (ca. 230 mg GAE/ g extract) but the water was the best solvent for extracting flavonoids (17.5 mg QE /g extract), while the higher results were obtained by Adaramola and Onigbinde (2016). Who approved the flavonoid contents of all the extracts of clove bud evaluated were higher than the phenolic

contents. The highest flavonoid content (501±0.58mg QE/g), phenolic content (200.2mgGAE/g),

**Table 2. Total phenolic and flavonoid compounds of Clove and Thyme different extracts.**

Extract	Phenolic compounds	Flavonoid compounds
	mg GAE/g <sup>a</sup>	mg QE/g <sup>b</sup>
Thyme		
E.E. whole	107.36	53.53
E.E. powder	140.17	66.11
Aq.E whole	46.50	126.50
Aq.E powder	54.05	111.16
E.O.E whole	125.35	101.51
E.O.E powder	158.83	64.16
Clove		
E.E. whole	367.44	141.88
E.E. powder	372.21	177.15
Aq. E whole	201.34	87.97
Aq. E powder	158.56	39.46
E.O.E whole	218.31	153.17
E.O.E powder	321.50	164.16

<sup>a</sup>(mg GAE/ g): mg of gallic acid <sup>b</sup>(mg QE/ g): mg of quercetin.

E.E= Ethanolic Extract, Aq = Aqua Extract, and E.O.E= essential oil Extract.

**3 antioxidants activist  
Reducing Power Assay**

Reducing power assay measures the total reducing capability of antioxidants on the basis of the extracts or vitamin C ability to reduce Fe+3 to Fe+2 ion. This assay treats the antioxidants in the samples as reductants in a redox-linked colorimetric reaction and is a relatively simple and easy procedure to be standardized. One possible disadvantage with this assay is the fact that this assay does not react fast with some antioxidants, such as glutathione. However, it is still suitable for the assessment of antioxidant activity of extract because only limited amounts of plant glutathione are absorbed by humans (Schafer and Buettner, 2001).

The analysis of reducing power capacity of herbal extracts indicated variable absorbance ratios in Table (3). The reducing power values of the ethanolic extract obtained from thyme-powdered leaves was the highest in all thyme extracts. The reducing power values of the ethanolic extract obtained from thyme-powdered leaves at 100 µg/mL was (1.022) comparable with vitamin C (8.911) at the same concentration (100 µg/mL). The essential oil extract obtained from clove-powdered buds was the highest in all clove extracts, which give (1.031) comparable with vit. C (8.911) at the same concentration (100 µg/mL). These results are agreed with those reported by Adaramola and Onigbinde (2016) who found that reducing the power of acetone extract was (0.88±0.10).

**Table 3. Reducing power capacity for different Clove and Thyme extracts concentrations.**

Herbal extract Concentration (µg/ml)	Thyme EE w	Thyme EE p	Thyme Aq. E w	Thyme Aq. E p	Thyme EO. E w	Thyme EO. EP	Clove EE w	Clove EE p	Clove E w	Clove Aq. E p	Clove EO. E w	Clove EO. EP	VIT C
25	0.4853	0.8048	0.2181	0.3085	0.7069	0.2353	0.8614	0.84	0.9685	0.295	0.7764	0.7985	2.4
50	0.5503	0.8773	0.2381	0.346	0.7269	0.2653	0.8814	0.87	0.9885	0.3	0.8464	0.876	4.571
75	0.6153	0.9498	0.2581	0.3835	0.7469	0.2953	0.9014	0.9	1.0085	0.305	0.9164	0.9535	6.741
100	0.6803	1.0223	0.2781	0.421	0.7669	0.3253	0.9214	0.93	1.0285	0.31	0.9864	1.031	8.911

E = Ethanolic Extract, Aq. = Aqueous Extract, E.O = essential oil Extract, W= whole, and P=powder

**The DPPH radical scavenging activity;**

There are various methods for the determination of antioxidant activities. The measurement of radical scavenging activity of any antioxidant is commonly associated with the use of the DPPH method because it is a quick, reliable, and reproducible method. It is widely used to test the ability of compounds as free radical scavengers or hydrogen donors and

to evaluate the antioxidative activity of extracts Martins *et al.* (2008). The stable radical DPPH has maximum absorption at 517 nm and the antioxidant reduces it to the yellow-colored diphenyl-picrylhydrazine

The antioxidant capacity of herbal extracts (scavenge DPPH free radical) were showed in Table (4). The obtained results showed that essential oil extract obtained from clove-

whole buds and essential oil extract obtained from thyme-whole leaves extracts had high free radical scavenging capacity (IC<sub>50</sub>; 2.75 and 2.86 µg/mL, respectively), compared with ascorbic acid (IC<sub>50</sub>; 6.75 µg/mL), considering that there

is a reverse relationship between the IC<sub>50</sub> value and the antioxidant activity. Clove oil shows powerful antioxidant activity (Chaieb et al. 2007).

**Table 4. Changes in DPPH radical scavenging activities according to different concentrations of Clove and Thyme extracts.**

IC%	Vit.C µg/ml	Thyme µg/ml						Clove µg/ml					
		EE w	EE p	Aq. E w	Aq. E p	EO. E w	EO. E P	EE w	EE p	Aq. E w	Aq. E p	EO. E w	EO. E P
40	5.16	56.93	55.28	178.01	190.90	1.33	3.89	19.39	11.69	8.45	8.24	1.27	1.61
50	6.75	78.27	78.86	226.30	257.90	2.86	5.48	50.76	45.49	30.15	36.51	2.75	4.21
60	8.33	99.61	102.45	274.76	324.89	4.40	7.07	82.13	79.29	51.85	64.78	4.22	6.81
70	9.92	120.95	126.04	323.13	391.89	5.93	8.66	113.50	113.10	73.55	93.05	5.70	9.42
80	11.51	142.29	149.63	371.50	458.88	7.47	10.25	144.87	146.90	95.25	121.32	7.17	12.02
90	13.10	163.63	173.22	419.87	525.88	9.00	11.84	176.24	180.70	116.96	149.59	8.65	14.62
100	14.69	184.97	196.80	468.24	592.87	10.53	13.43	207.61	214.51	138.66	177.87	10.12	17.23

E = Ethanolic Extract, Aq. = Aqueous Extract, E.O = essential oil Extract, W= whole, and P=powder

**4 Antibacterial activity of different clove and thyme extracts**

The antibacterial activity of extracts at different concentrations was determined using the well agar diffusion method against three pathogenic bacteria. The diameters of inhibition zones were measured and taken as an indicator of inhibition activity. Table (5) shows that thyme essential oil extract obtained from thyme-powdered leaves had gave the highest inhibition zone against *Escherichia coli*, and no inhibition zone was observed in the aqueous extract obtained from thyme-powdered leaves. Essential oil extract obtained from thyme-whole leaves presented the highest inhibition zone against *Staphylococcus aureus* and no inhibition zone was observed in the ethanolic extracts of thyme and aqueous extract obtained from thyme-powdered leaves but, the same extract presented the inhibition zone against *Bacillus cereus*. These results are in the same trend with those of Choulitoudi et al. (2016) and Farag et al. (2019) who indicated that the essential oil of thyme had both antimicrobial and antioxidant activity which could be mainly attributed to its high content in carvacrol-Terpinene.

**Table 5. Antibacterial activity of clove and thyme different extracts**

Extract	E. coli	Staph.	Bacillus
Thyme			
EE whole	13.50	0.00	0.00
EE powder	7.50	0.00	9.25
Aq. E whole	8.25	11.50	13.25
Aq. E powder	0.00	0.00	14.50
EOE whole	8.75	24.50	10.25
EOE powder	19.75	12.25	14.00
Clove			
EE whole	14.50	10.50	13.25
EE powder	12.75	12.00	13.50
Aq. E whole	10.50	10.00	13.25
Aq. E powder	13.50	10.50	13.00
EOE whole	12.50	12.25	13.00
EOE powder	11.00	0.00	12.75

EE= Ethanolic Extract, Aq.= Aqua Extract, and E.O.E= essential oil Extract.

In clove extracts, the ethanolic extract obtained from clove-whole buds presented the highest inhibition zone against *Escherichia coli*, and the lowest inhibition zone was observed in the aqueous extract obtained from clove-whole buds. While essential oil extract obtained from clove-whole buds presented the highest inhibition zone against *Staphylococcus aureus* and no inhibition zone was observed in the essential oil extract obtained from clove-powdered buds.

Against *Bacillus cereus*, the highest inhibition zone was observed in the ethanolic extract obtained from clove-

powdered buds. This antibacterial activity might be due to its content of total phenolic and flavonoids compounds (Choulitoudi et al. 2016). These results in agreement with Nzeako et al. (2006) who reported that the thyme and clove oil extracts are known to possess some antimicrobial activities and it may be due to terpenes (thymol and eugenol) flavones, glycosides of phenolic monoterpenoids and aliphatic alcohols among other compounds.

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### النشاط المضاد للأوكسدة والبكتريا لمستخلصات القرنفل (*Syzygium aromaticum*) والزعر (*Tymus vulgaris*)

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في الأونة الأخيرة، هناك اهتمام متزايد باستخدام المواد الكيميائية النباتية المضادة للأوكسدة الطبيعية لتجنب آثار مضادات الأوكسدة الصناعية ومضادات الميكروبات. في هذا البحث، تم استخدام عشبين، وهما القرنفل والزعر، لتقدير المركبات النشطة بيولوجيا. تم تقدير التركيب الكيميائي لبراعم القرنفل وأوراق الزعر. تم إجراء عمليات الاستخلاص الإيثانولية والمائية للحصول على مركبات نشطة بيولوجية. وقد أظهرت النتائج احتواء مستخلصات براعم القرنفل على أعلى نسبة إجمالية من المركبات الفينولية TPC (372.21 مجم من مستخلص GAE / جم) بينما كانت أوراق الزعر المطحونة (158.83 مجم GAE / جم مستخلص). كان إجمالي محتوى الفلافونويد (TFC) لبراعم القرنفل المسحوق (177.15 مجم QE / جم مستخلص) لكن أوراق الزعر الكاملة كانت (126.50 مجم QE / جم مستخلص). كانت القوة المختزلة لأوراق مسحوق الزعر (1.022) مقارنة بفيتامين سي (8.911) بينما كانت براعم القرنفل (1.031). كانت سعة الكسح الجنور الحرة لبراعم القرنفل الكاملة (IC<sub>50</sub>؛ 2.75 ميكروغرام / مل) وكانت مستخلصات أوراق الزعر الكاملة (IC<sub>50</sub>؛ 2.86 ميكروغرام / مل)، مقارنة بحمض الأسكوربيك (IC<sub>50</sub>؛ 6.75 ميكروغرام / مل). تم تقدير النشاط المضاد للبكتيريا لجميع مستخلصات الأعشاب المستخدمة ضد *Escherichia coli* و *Staphylococcus aureus* و *Bacillus cereus* وقد أظهرت النتائج ان الزيت العطري لاوراق الزعر المطحون على *Escherichia coli* كان (19.75 mm منطقة تثبيط) بينما كان المستخلص الإيثانولي لبراعم القرنفل الكاملة (14.5 mm منطقة تثبيط). وكان زيت أوراق الزعر الكامل (24.5 mm منطقة تثبيط) ضد *Staphylococcus aureus*، لكن مستخلص الزيت العطري لبراعم القرنفل الكاملة كان (12.25 mm منطقة تثبيط). المستخلص المائي المستخلص من أوراق الزعر المسحوق كان (منطقة تثبيط 14.5 مم) بينما كان المستخلص الإيثانولي لبراعم القرنفل (منطقة تثبيط 13.5 مم) ضد *Bacillus cereus*