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Phytochemical Analysis, Antibacterial and Antioxidant Activity Study of *elettaria Cardamomum*seed

Loh Kok Shen, Nabila Perveen, Naeem Hasan Khan
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, AIMST University, Bedong, Kedah D.A., Malaysia.
Corresponding author: Prof. Dr. Naeem Hasan Khan
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ABSTRACT: To evaluate the phytochemical constituents, antimicrobial and antioxidant scavenging activity of ethanolic Soxhlet and maceration extracts. In present research, soxhlet extraction and maceration extractions were applied to the seed of *Elettaria Cardamomum*by using absolute ethanol.Phytochemical analysis for the important chemical constituents from ethanolic extract were carried out.Antimicrobial activity of *Elettaria Cardamomum*extract was carried out using well diffusion method on the Nutrientagar.Antioxidant activity of *Elettaria Cardamomum*was carried out using total phenolic content test and DPPH to identify the percentage of scavenging by the chemical constituents. For phytochemical analysis, only test for alkaloids, test for reducing sugar, test for saponin, test for salkowski'test and test for carbohydrates that showed positive results.For antibacterial screening, all the concentrations of showed negative results due to low concentration of extract being used.For antioxidant analysis, total phenolic contents and DPPH radical scavenging showed antioxidant result for *Elettaria Cardamomum.*It is concluded that *Elettaria Cardamomum*is a very essential plant medicinally. A long term research project is a must to evaluate the pharmacological uses of extracts and differences of solvents that can be used to isolate the pure and high yield of chemical constituents from the plant.
KEYWORDS: Soxhlet, maceration, antibacterial, antioxidant, phytochemical.

I. INTRODUCTION

*Elettaria cardamomum*has been consumed as food spice worldwide since ancient time. The plant, flowers and fruits (seeds) of *Elettaria cardamomum* are shown in Figures 1-4. [(1)]

Figures 1-4: *Elettaria Cardamomum*plant, flower and its fruit (seeds).

Green Cardamom (*Elettaria cardamomum*) is an aromatic spice that mainly cultivated in Southern India, Sri Lanka, Southeast Asia, Guatemala and in Ceylon.[(2)]It is called as the "Queen of spices" which it is a tall, perennial, and reed–like herb growing wild. Cardamom is one of the world's ancient, highly valued spices and expensive. Its fruits are in oblong shape and gray in color and each containing many seeds. Its functions are used for against cardiac disorders, renal and vesicular calculi, asthma and gastrointestinal disorders including

indigestion and flatulence. [3,4]The taxonomical classification of *Elettaria Cardamomum* is shown in Table 1. Table 2 shows the different linguistic names of *Elettaria Cardamomum* around the globe.

Table 1Taxonomical classification of *Elettaria Cardamomum*

Taxanomy	Classification
Kingdom	Plantae
Subkingdom	Tracheobinta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Genus	Elettaria Maton
Species	*Elettaria Cardamomum* (L) Maton

Table 2Different language of *Elettaria Cardamomum*[

Arabic	**هيل, هال, حب بهان, الهان حب, الهال حب** **هَيل, هَال, الْهَان حَبُّ, الْهَال حَبُّ, حَبَّهَان** **Habbahan, Habbu al-hal, Habbu al-han, Hail, Hayl, Hal**
Chinese (Cantonese)	白豆蔻 [baahk dáu kau], 小豆蔻 [síu dáu kau]
English	Green Cardamom
French	Cardamome (vert)
German	(Grüner) Kardamom, (Grüner) Cardamom
Japanese	カルダモン, ショウズク Karudamon, Shōzuku, Shozuku
Korean	카다몬, 카도몬, 소두구, 백두구 Kadamom, Kadomom, Sodugu; Paektugu, Baegdugu
Malayalam	ഏലം, ഏലക്ക, ഏലക്കായ് Aelakka, Aelakkayu, Aelam, Elathari
Portuguese	Cardamomo
Tulu	ಏಲಕ್ಕಿ Elakki
Hindi	Elathi
Urdu	Elachii

For macroscopic characteristic of *E. cardamomum* plant which it has a tuberous horizontal rhizome. It is simple, smooth, green and shining, perennial stems, which rise from 6-12 feet in height, and bear alternate elliptical-lanceolate sheathing leaves. The flower-stalk proceeds from the base of the stem, and lies upon the ground, with the flowers arranged in a panicle. The fruit is a three-celled capsule that containing many seeds but during drying it loses three-fourths of its weight.[5]The Phytochemical analysis of *E.cardamomum* in methanol extract by different researchers is laid down in Table 3. [6,7]

Table 3Phytochemical analysis of *E.cardamomum* methanol extract

Phytochemicals	***E.cardamomum*** **(methanol extract)**
Alkaloids	-
Flavonoids	+
Phenol	-
Glycoside	+
Saponins	-
Quinone	-
Tannis	-
Terpenoids	+

RATIONAL STUDY

α-terpinyl acetate is the active constituent present in *E.Cardamomum.*[36], *as* the main ingredient of this plants species which it is a flavouring agent. This research study was carried out to discover the antibacterial and antioxidant activity of *Elettaria cardamomum* therapeutically. Thus, this research was started to identify and to evaluate the potential antibacterial, antioxidant and phytochemical screening of the fruits (seeds)extracts of *Elettaria cardamomum.*

OBJECTIVE

To analyse phytochemical constituents from the fruits (seeds) of *Elettaria cardamomum*using suitable solvents and extraction technique and to evaluate the *in-vitro* antibacterial and *in-vitro* antioxidant activities of fruits (seeds)extract of *Elettaria cardamomum.*

II. METHODOLOGY AND EXPERIMENTAL

The seeds were separated, manually, from the outer skull. Then the seeds were air washed, dried and blended. The cardamom seed is then poured into the blender to blend it into smaller size. The blended cardamom seeds weight was 429.00 g.

The following chemicals were required for the phytochemical screening:

Absolute Ethanol (John Kollin Corporation, USA), Sulphuric Acid (95-99%) (EMD M.C. Germany), Hydrochloric Acid (37%) (Fnendemann Schmidt Chemical), Fehling's A and Fehling's B reagent (R&M Chemicals, UK), Sodium Hydroxide (Friendemann Schmidt Chemical), Distilled water freshly prepared in the laboratory, Chloroform (EMD M.C. Germany), Dragendorff;s reagent (R&M Chemicals, UK), Molisch's reagent (R&M Chemicals, UK), Ferric Chloride (EMD M.C. Germany), Lead acetate and Ammonia.

The following chemicals were required for the antibacterial activity determination: Extracts obtained from maceration extraction and Soxhlet extraction, Distilled water fleshly prepared in the laboratory, 90% ethanol freshly distilled in the laboratory. (John Kollin Corporation. USA), LB Broth, LAB lvt., Nutrient Agar, Nutrient Broth.

Bacterial Strains

- *Bacillus subtilis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Staphylococcus aereus*

The following chemicals were required for the antioxidant activity determination:

Ethanol 95% (John Kollin Corporation, USA), Methanol (J.T. Baker. Center Valley, PA), 2,2-Diphenyl-1-picryhydrazyl (DPPH) reagent. Sisco Research Laboratories P\,1. Ltd. India), Butyl Hydroxyl Toluene (BHT). Nacalai Tesque, Inc. Japan, Sodium bicarbonate. (Bendosen), Folin-Ciocalteu's Phenol reagent (EMD M.C. Germany), Gallic acid. (R&M Chemicals, UK), Distilled water freshly prepared in the laboratory, Extracts obtained from Soxhlet extraction.

Maceration extraction using ethanol

It is an extraction process that consists of maintaining in contact between the solvent and the plant or extract for a period of time and conducted. Before start this extraction, the extract should be in fresh or dry form.[37]Generally, alcohol will be the most used substance because it can extract a greater part of molecules

such as active ingredients which contained within the plant that including molecules which are hydrophilic or lipophilic. [37]

300g of the blended cardamom seeds wereweighed and poured into the conical flask. Then, the 500ml of absolute ethanol (solvent) was added into the conical flask containing blended cardamom seeds powder.The mixture of ethanol and cardamom was swirled for 4-5 minutes about 10 times a day for 7 days at room temperature. The mixture was filtered by using water vacuum system. The maceration extract was concentrated over rotary evaporator for around 2 hours. The rotation per minutes was kept at around 100 and the temperature was 65° C. The total amount recovered was 190 ml. Then this semi solid mass was further dried over water bath for 24 hours at a temperature of 70° C.[38]

Soxhelt extraction using ethanol

300.00 g of the blended cardamom seeds were weighed and placed inside a thimble of Soxhelt apparatus. 350 ml of ethanol was used for the extraction and poured into the round bottom flask and heated.The heating / boiling was continued throughout the process until the liquid of the extract at siphon tube is colourless. The whole process took about 24 hours. The recovery of the extract was 250 ml. This volume was then concentrated by using rotary evaporator for around 2 hours. The rotation per minutes was around 100 and the temperature was kept at 65° C. After that poured in the petri dish and put on it to let it be more concentrated till it became gummy. The petri dish was covered by aluminium foil.Make some holes on the surface of aluminium foil so that evaporation can occur. It took around 3-5 days to let the extract completely dry. The temperature of water bath is at 70° C. The weight of concentrated Soxhlet extract after water bath drying was4.5g.[39]

PHYTOCHEMICAL SCREENING

Various medicinal properties have been attributed to natural herbs in which the medicinal plants constitute main source of new pharmaceuticals and healthcare products.[40,41]Phytochemical screening is refer to extraction, screening and identification of the medicinally active substances that found in the plant and some of the bioactive substance can derived from plant was such as flavonoids, alkaloids, carotenoids, tannins, antioxidants and phenolic compounds. Secondary metabolites of plant serve as defense mechanism against predation by many microorganism, insects and herbivores. [41]Macerated and Soxhelt extracts were used in phytochemical screening tests.

Alkaloids

1.0 ml of the each extract warmed with 2.0 ml of 2.0% Sulfuric Acid for 2 minutes. It was filtered and few drops of Dragendoff's reagent were added.

Reducing Sugars

1.0 ml of each extract were shaken with 1 ml of distilled water and filtered. Filtrate boiled with few drops of Fehling's solution A and B (Equal volume of Fehling's A and Fehling's B was mixed) for few minutes.

Saponins

1.0 ml of each extract added with 5.0 ml of distilled water and heated to boil.

Terpenoids or Steroids (Salkowski Test)

1.0 ml of each extract was mixed with 2.0 ml of chloroform ($CHC1_3$) and 3.0 ml concentrated Sulfuric Acid (H_2SO_4) was carefully added to form a layer.

Antraquinones

1.0 ml of each extract was boiled with 2.0 ml of 1O.O% Hydrochloric Acid (HCl) solution for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform ($CHCI_3$) was added to filtrate. Few drops of 10.0% NH3 were added to mixture and heated.

Glycosides

1.0 ml of each extract was hydrolyzed with 1.0 ml of 10.0% Hydrochloric Acid (HCl) solution and neutralized with 1.0 ml of 0.2M Sodium Hydroxide (NaOH) solution. A few drops of mixture of Fehling's solution A and B were added.

Tannins

1 .0 ml of each extract was mixed with 2.0 ml of distilled water and heated on water bath. The heated mixture filtered and 1ml of ferric chloride added to filtrate.

Flavonoids

1.0 ml of each extract was dissolved in 1.0 ml of diluted 0.2 M Sodium Hydroxide (NaOH) and 1.0 ml of 10% Hydrochloric Acid (HCl) solution was added.

Carbohydrates

0.2 ml of Molisch's Reagent was added to 1.0 ml of each extract in a small test tube. After mixing, the tube was tilted. Without stirring, 0.5 ml of concentrated Sulfuric Acid (H_2SO_4) was carefully added by pouring down the side of the test tube.

Phenols

Few drops of 10.0 % Lead Acetate solution was added to 1.0 ml of each extract in a small test tube.

ANTIBACTERIAL ACTIVITY

Well agar diffusion method

Antimicrobial activity can be determined by using agar diffusion method. Agar diffusion is a method where the specific microorganism are inoculated at specific temperature. Discs containing different antibiotics were placed containing microorganism. The zone of inhibition formed will be measured by measuring the diameter. The diameter was compared with the according to the standard to identify the potential of the antimicrobial activity of the antibiotics.(42)

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial that will inhibit or prevent the visible growth of a microorganism after overnight incubation. MIC are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore to use for judging the performance of all other methods of susceptibility testing. MIC are used for diagnostic laboratories to confirm unusual resistance to give a definitive answer when a borderline result is obtained by other methods of testing or when disc diffusion methods are not appropriate. It also used to determine in vitro activity of new antimicrobial and MIC breakpoints. (43)

Minimum bactericidal concentration (MBC)

MBC is the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media. MBC determinations are undertaken less frequently and their major use has been reserved for isolates from the blood of patient with endocarditis. (43)It can be determined from the broth dilution of MIC tests by sub-culturing to agar plates that do not contain the test agent. The MBC is complementary to the MIC. MBC testing can be a good and relatively inexpensive tool to simultaneously evaluate multiple antimicrobial agents for potency.(43)

Serial Dilution of macerated extract

The 100mg/ml of extract was prepared as a stock solution.4000mg of macerated extract was weighed and was dissolved with 40ml of DMSO (Dimethyl sulfoxide).Sonicator was used to make sure the extract is completely dissolved in the DMSO.

1. 10ml of 100mg/ml of stock solution was used to prepare 100mg/ml of extract
2. 8ml of 100mg/ml of stock solution was mixed with 2 ml of DMSO to prepare 80mg/ml of extract
3. 6ml of 100mg/ml of stock solution was mixed with 4 ml of DMSO to prepare 60mg/ml of extract,
4. 4ml of 100mg/ml of stock solution was mixed with 6 ml of DMSO to prepare 40mg/ml of extract.
5. 2ml of 100mg/ml of stock solution was mixed with 8 ml of DMSO to prepare 20mg/ml of extract
6. 1ml of 100mg/ml of stock solution was mixed with 9 ml of DMSO to prepare 10mg/ml of extract.

Preparation of 4 bacterial strains

1. 100ml of sterilized nutrient broth was prepared by dissolving in a 250ml.
2. *Staphylococcus aureus* inoculate in the nutrient broth with 3 - 4 full loop.
3. The nutrient broth with bacterial strain was incubated at a temperature of 37^{o}C for 24 hours.
4. Step 1 to step 3 was repeated with *Bacillus Subtilis*, *Escherichia Coli*, and *Pseudomonas aeruginosa.*

Preparation of nutrient agar plate

1. 28g of nutrient agar powder was weighed.
2. The nutrient agar powder was dissolved in 625ml of distilled water.
3. After stirring, nutrient agar solution was subjected to autoclave for sterilization at temperature of 121^{o}C for 2 hours.
4. The hot sterilized nutrient agar solution was poured into the petri plate in laminar air flow cabinet.
5. Each of the petri plate contained approximately 25ml of nutrient agar solution which can only occupied 60-70% of the petri plate.
6. The nutrient agar solution in the plate was allowed to solidify.

Agar Diffusion Test

1. The content that was labeled is name, NA (Nutrient Agar), date, extract, bacterial strain and concentration.
2. 0.1ml of nutrient broth containing bacterial strain was transferred on the plate.
3. The sterilized L- shaped spreader was used to spread the bacterial strain on the plate evenly. Before spreading, the spreader was sprayed by alcohol and introduced on to the flame to kill any contaminant.
4. 3 wells were made on the plate by using sterilized borer. The 3 wells must be separated with each other evenly forming anequal triangular distance.

5. Then different concentration of extract was transferred into the well according to the label by using micropipette. The well was filled until around 60% of the well's height.
6. The plate with bacterial strain and extract was incubated at temperature of 37^{o}C for 24 hours.
7. The zone of inhibition was observed and recorded.
8. For each bacterial strain, 4 plates were needed for 2 duplicate set. 1 plate was used to make positive control, and 1 plate was used for negative control plate(all bacterial strain shared one)

MIC

Preparation of standard McFarland bacteria culture

1. 4-5 loops of the bacterial strains were cultured in each sterile nutrient broth

incubated at 37° C for 24 hours and centrifuged at 5000rpm for 10 minutes

2. The supernatant was discarded and the resulting cell mass was re-suspended in another new sterile nutrient broth
3. The suspension was standardized by ensuring an absorbance reading range is around 0.08-0.1 using a spectrophotometer at 625nm.

MIC test

1. 30mg of the dried gummy macerated extract was dissolved in 3ml of DMSO to obtain concentration of 1000µg/ml.
2. Two fold serial dilutions were made to get 5 different concentration which was 1000, 500, 250, 125, and 62.5µg/ml.
3. 0.2ml of extract solution was transferred into a cleaned assay tube by micropipette and 1.8ml of standard McFarland bacterial culture was then added into the tube containing extract solution by micropipette
4. 1ml of the mixture was then transferred into a second cleaned assay tube already containing 1ml of standard McFarland bacteria culture by micropipette to obtain 500µg/ml. The steps were repeated until 62.5µg/ml concentration was achieved.
5. Each time transfer, the micropipette tip should be changed. This is to prevent the mixing of concentration or increase in concentration.
6. The process was repeated for each bacterial strain and duplicate sets were made.
7. Four positive tubes containing only the bacterial culture suspension were prepared respectively.
8. One negative tube containing only the sterilized nutrient broth was also prepared.
9. The tubes were then incubated at 37^{o}C for 24hours.
10. The MIC activity was observed and determined.

MBC

1. After 24 hours incubate, the tube which showed clear solution was chosen as test sample for each bacterial strain.
2. The agar plate was labeled with name, Nutrient agar, name of bacteria and date
3. 0.1ml of incubated nutrient broth of selected tube was transferred onto an agar plate and spread evenly
4. 0.1ml of each clear solution of bacterial strain was transferred onto an agar plate and spread evenly.
5. The plate was incubated at 37^{o}C for 24hours.
6. The result was observed.

ANTIOXIDANT(DPPH**,**2,2-diphenyl-1-picryl-hydrazyl-hydrate)

They are sometimes called "free-radical scavengers."The sources of antioxidants can be natural or artificial. Certain plant-based foods are thought to be rich in antioxidants. Plant-based antioxidants are a kind of phytonutrient, or plant-based nutrient.[45]

It is a stable free radical on room temperature and appears as violet solution in alcohol. On the accepting proton from a corresponding donor which is antioxidant molecule, it gets reduced and decolorize from deep purple to yellow color. The evaluation of the antioxidant activity of sample was determined based on the scavenging activity against the free radical DPPH through the calculation of IC_{50}, which represents the concentration of the material in question necessary to inhibit 50% of free radicals. Thus the lower IC_{50} value in a particular sample indicates a greater ability to neutralize the free radicals. The DPPH contains an odd electron, which is responsible for the absorbance at 514nm and also for a visible deep purple color[47]Hence in this study, absorbance of both sample and BHT(Butylated hydroxytoluene) standard were measured at wavelength of 514nm. Standard calibration curve was plotted with an equation of y= 0.2954x + 4.3016,R2= 0.945.[46]

TPC(Total phenolic content)

The Folin-Ciocalteu (FC) reaction is an antioxidant assay based on electron transfer, which measures the reductive capacity of an antioxidant. The FC reagent uses a mixture containing sodium molybdate, sodium tungstate, and other reagents. When produces a blue colour which absorbs at 750nm, it indicated that the phenols is present. Therefore, the higher the absorbance produced, higher the total phenolic content. It is also called as Gallic Acid Equivalent Method (GAE) as Gallic acid is used as the standard in this method.[48]

Preparation of extract and standard

1. The soxhlet extract was used in antioxidant.
2. Six different concentration of extracts were prepared at 10, 20, 40, 60, 80, 100 µg/ml.
3. Serial dilution method was used to prepare different concentration.
4. 10mg of gummy extract dissolved in 10ml of ethanol to prepare a stock solution of 1mg/ml concentration.
5. From the stock solution, 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1ml was pipette out and made up to 10ml individually to produce a concentration of 100 µg/ml, 80 µg/ml, 60 µg/ml, 40 µg/ml, 20 µg/ml, and 10 µg/ml respectively.
6. Butylated hydroxyltoluene (BHT) was used as standard in this assay.
7. Six different concentration of extracts were prepared which were 10, 20, 40, 60, 80, 100 µg/ml.
8. The method of serial dilution is the same as the preparation of the extracts.

Procedure

1. The mixture was kept in the dark place at room temperature for 30minutes and the absorbance was measured at 518nm using a spectrophotometer.
2. The same procedure was repeated by replacing the extracts with different concentration of BHT to determine the standard graph.
3. The control absorbance value is determined by repeating the same procedure with replacement of the extracts with 2.5ml of ethanol.
4. Sample measurement was done once and the result was calculated.
5. The anti-oxidant activity was determined by following formula:

Percentage of Anti-oxidant Activity (%) = [(Control absorbance – Sample absorbance)/Control absorbance] x 100%

TPC

Preparation of the Extracts and Standard

1. The extracts were obtained from soxhlet extraction.
2. Five different concentration of extracts was prepared that are 25µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml.
3. 2mg of extract was dissolved in 10ml of ethanol stock solution of 200 µg/ml concentration.
4. Then it was double diluted by diluting 2ml of stock solution with 2ml of ethanol to obtain concentration of 100µg/ml.
5. The same procedure was repeated by taking 2ml from 100µg/ml and diluting with 2ml of ethanol to obtain a concentration of 50µg/ml.
6. 1ml of extract was taken from 50 g/ml and diluting with 2ml of ethanol to get 25 µg/ml concentration of extract.
7. Gallic acid was used as the standard for this assay. Five different concentration was prepared which is 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 µg/ml.

Procedure

1. For every 0.2ml of extract, about 0.2ml of Folin-Ciocalteu reagent and 4ml of 2.5% sodium carbonate were added and allowedto stand for 2hours.
2. Then the absorbance of the solution at 750nm was measured.
3. The same procedure was repeated by replacing the extract with the Gallic acid.

III. RESULTS

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The results of phytochemical screening are shown in Table 4

Table 4The result of phytochemical screening

S.NO	TEST	SOXHLET	MACERATION
1	Alkaloid Test	+	-
2	Reducing Sugar Test	+	+

3	Saponin Test	+	+
4	Salkowski Test	+	+
5	Anthraquinone Test	-	-
6	Glycoside Test	-	-
7	Tannin Test	+	-
8	Flavonoid Test	-	-
9	Carbohydrate Test	+	+
10	Phenol Test	-	-

-: negative, +: positive

ANTIBACTERIAL ACTIVITY

Well diffusion test.

The zone of inhibition of *Bacillus subtilis* and *Escherichia coli* in mm is shown in Table 5.

Table5Zone of inhibition of *Bacillus subtilis* and *Escherichia coli* in mm.

Concentration (mg/ml)	**Diameter of Zone of Inhibition (mm)**					
	Bacillus subtilis			*Escherichia coli*		
10	1st set	2nd set	average	1st set	2nd set	average
	5	-	2.44	-	-	-
	5	-		-	-	
	6	-		-	-	
	5	-		-	-	
	4	-		-	-	
	5	-		-	-	
	5	-		-	-	
	4	-		-	-	
Average	4.88					
20	5	-	2.25	-	-	-
	4	-		-	-	
	4	-		-	-	
	5	-		-	-	
	4	-		-	-	
	5	-		-	-	
	5	-		-	-	
	4	-		-	-	
Average	4.5					
40	5	2.5	2	-	5	2.88
	2.5	5		-	7	
	2.5	3		-	5	
	5	4		-	6	
	5	2.5		-	5	
	3	5		-	7	
	4	6		-	6	
	5	4		-	5	
Average	4	4		-	5.75	
60	10	11	12	23	7	14.32

	9	17		23	6	
	10	14		20	7	
	10	14		19	7	
	11	13		21	14	
	10	15		20	7	
	10	15		22	6	
	10	13		20	7	
Average	10	14		21	7.63	
80	10	10	10.94	20	1	10.63
	10	11		20	2	
	8	12		19	1	
	12	15		19	2	
	10	10		20	3	
	11	13		20	1	
	9	11		20	1	
	10	13		19	2	
Average	10	11.88		19.63	1.63	
100	13	10	11.07	5	5	4.13
	12	11		5	3	
	9	10		4	5	
	10	12		4	3	
	13	10		5	4	
	12	11		5	3	
	11	10		5	3	
	13	10		4	3	
Average	11.63	10.5		4.63	3.63	

***- :** no zone of inhibition

Figure5 Positive and control of*Bacillus*

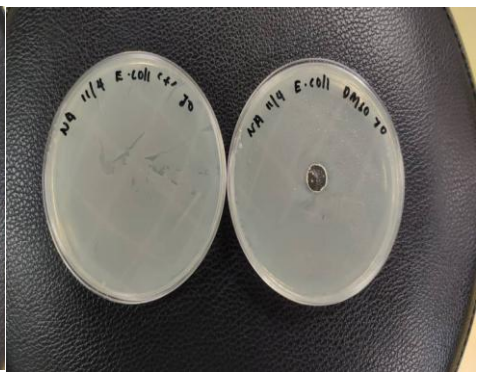


Figure 6 Positive and control of*subtilis (B.Subtilis)Escherichia coli (E.coli)*

Table6shows the zone of inhibition of *Pseudomonas Aeruginosa* and *Staphylococcus Aereus* in mm.

Table6Zone of inhibition of *Pseudomonas Aeruginosa* and *Staphylococcus Aereus* in mm.

Concentration (mg/ml)	Diameter of Zone of Inhibition (mm)					
	Pseudomonas Aeruginosa			*Staphylococcus Aureus*		
10	1st set	2nd set	average	1st set	2nd set	average
	-	-	-	10	11	11.38
	-	-		11	12	
	-	-		10	10	
	-	-		11	15	
	-	-		12	12	
	-	-		10	11	
	-	-		12	12	
	-	-		10	13	
Average				10.75	12	
20	-	-	-	10	7	10.19
	-	-		7	8	
	-	-		12	12	
	-	-		11	15	
	-	-		10	9	
	-	-		10	13	
	-	-		10	7	
	-	-		11	11	
Average				10.13	10.25	
40	-	-	-	15	16	15.82
	-	-		15	15	
	-	-		17	18	
	-	-		15	18	
	-	-		16	15	
	-	-		15	14	
	-	-		18	14	
	-	-		17	15	
Average	-	-		16	15.63	
60	-	-	-	3	15	8.8
	-	-		3	12	
	-	-		5	15	
	-	-		2	16	
	-	-		4	13	
	-	-		3	15	
	-	-		4	15	
	-	-		2	14	
Average	-	-		3.25	14.34	
80	-	-	-	5	14	8.26
	-	-		5	12	

	-	-		3	10	
	-	-		3	11	
	-	-		5	12	
	-	-		5	12	
	-	-		4	11	
	-	-		5	15	
Average	-	-		4.38	12.13	
100	5	5	4.26	5	1	2.75
	4	3		2	2	
	4	4		3	3	
	4	5		2	3	
	5	5		2	2	
	5	5		5	3	
	3	4		3	3	
	5	4		2	3	
Average	4.13	4.38		3	2.5	

***- :** no zone of inhibition

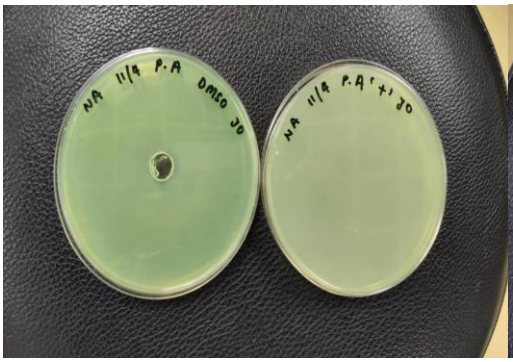


Figure7 Positive and control of *Pseudomonas Aeruginosa (P.Aeruginosa)*

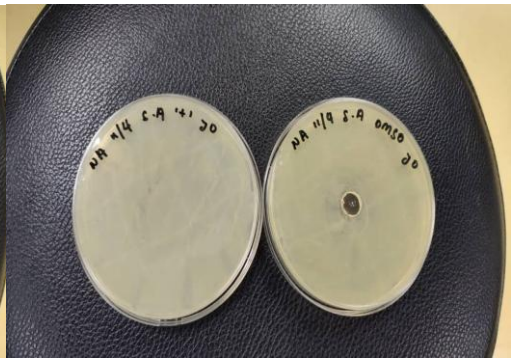


Figure8Positive and control of *Staphylococcus Aureus (S.Aureus)*

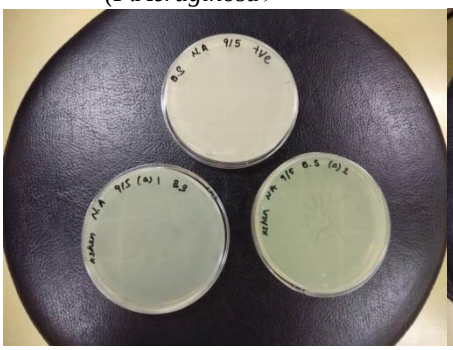


Figure9MBC for **set 1** of *B.Subtilits*with positive control

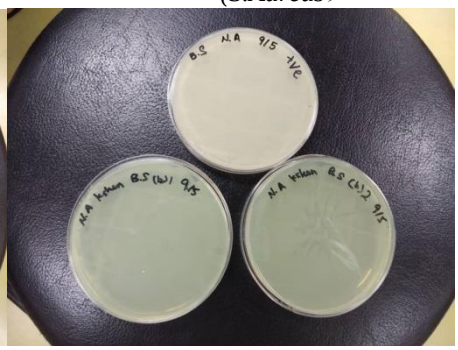


Figure 10 MBC for **set 2** of *B.Subtilits* with positive control

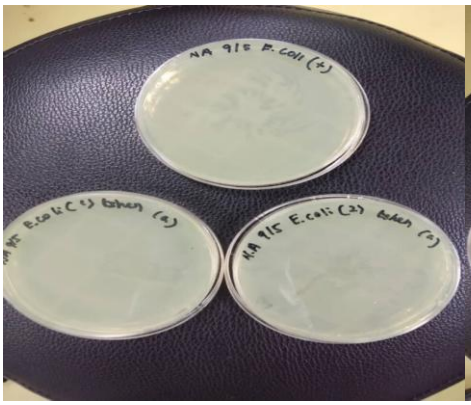
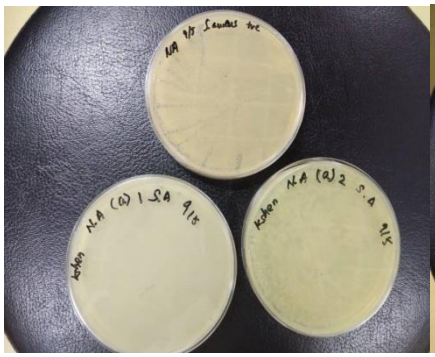


Figure 11 MBC for **Set 1** of *Escherichia* Figure 12 MBC for **Set 2** of *Escherichia(E.coli)* with positive control*(E.coli)* with positive control



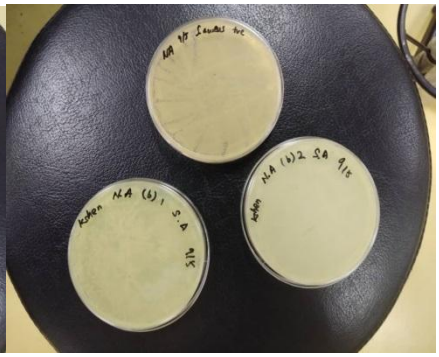
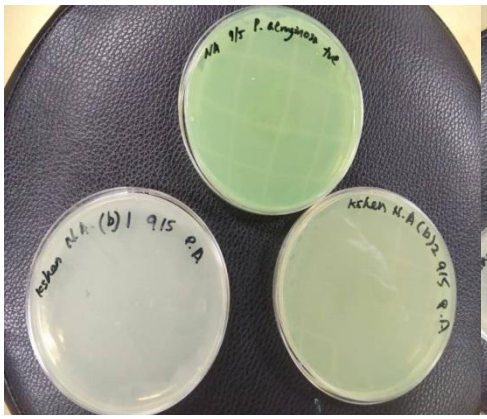


Figure 13MBC for **Set 1** ofFigure 14 MBC for **Set 2** of *StaphylococcusAureusStaphylococcusAureusS.Aureus*） with positive control (*S.Aurues)*with positive control



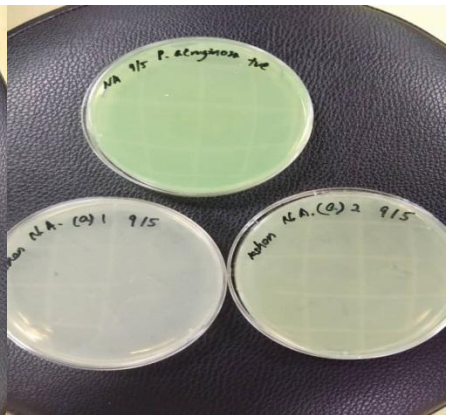


Figure15MBC for **Set 1** ofFigure 16 MBC for **Set 2** of *Pseudomonas AeruginosaPseudomonas Aeruginosa* (*P.Aeruginosa*）with positive control (*P.Aeruginosa*）with positive control

ANTIOXIDANT

DPPH scavenging activity

Concentration and corresponding absorbance of BHT is shown ion Table 7.

Table7 Concentration and corresponding absorbance of BHT

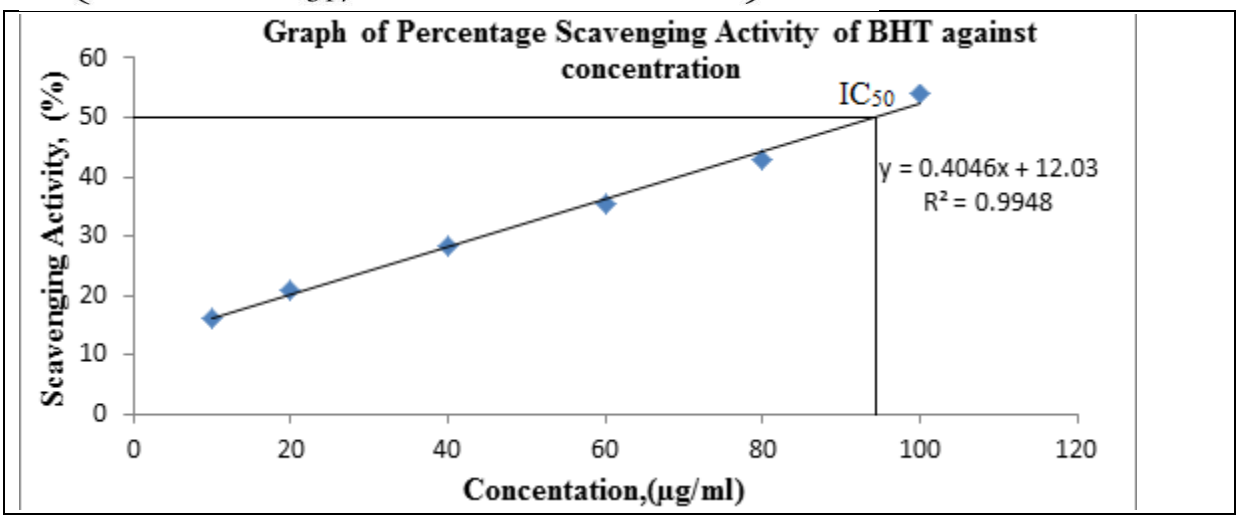
Concentration (**µg/ml**)	Absorbance Values	Scavenging Activity (%)
10	0.488	16.15
20	0.461	20.79
40	0.418	28.18

60	0.375	35.57
80	0.332	42.96
100	0.268	53.95

*control= 0.582

The scavenging activity is determined by the following formula:

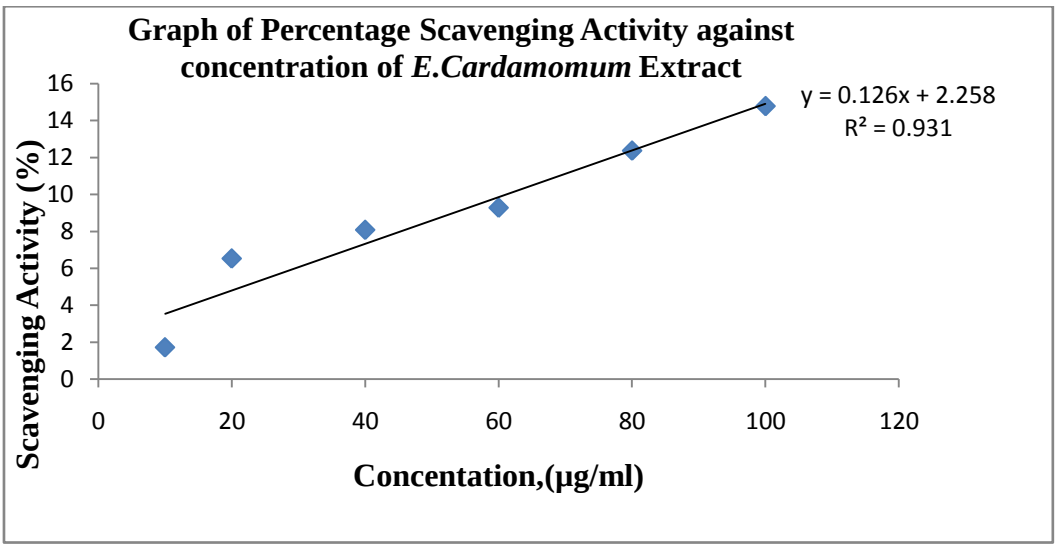
$$\text{Scavenging activity}(\%) = \left(\frac{A_{517}\ \text{of control} - A_{517}\ \text{of sample}}{A_{517}\ \text{of control}}\right) \times 100$$



Graph1Percentage Scavenging Activity of BHT

Table 8Concentration and corresponding absorbance of *E.Cardamomum soxhlet* extract

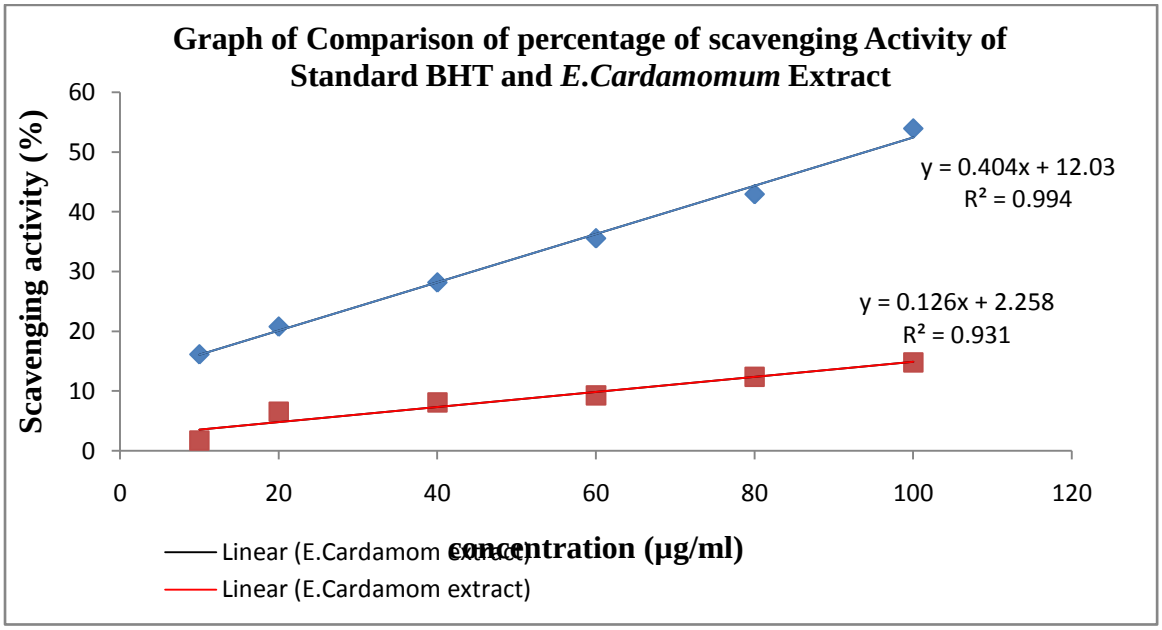
Concentration (**µg/ml**)	Absorbance **Values**	Scavenging Activity (%)
10	0.572	1.72
20	0.544	6.53
40	0.535	8.08
60	0.528	9.28
80	0.510	12.37
100	0.496	14.78



Graph 2 Concentration and corresponding absorbance of *E.Cardamomum soxhlet* extract

Table 9: Comparison of scavenging activity of BHT and *E.cardamomum*

Concentration (**µg/ml**)	Scavenging Activity (%)	
	BHT	***E.Cardamom***
10	16.15	1.72
20	20.79	6.53
40	28.18	8.08
60	35.57	9.28
80	42.96	12.37
100	53.95	14.78

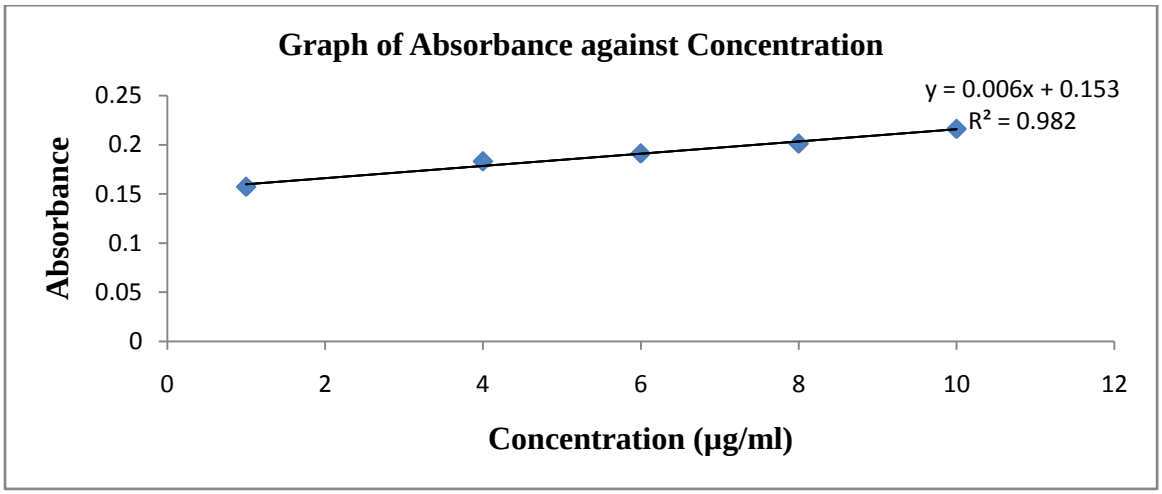


Graph 3 Comparison of percentage scavenging activity of standard BHT and *E.Cardamomum*extract.

TOTAL PHENOLIC CONTENT

Table10Concentration and corresponding absorbance of standard Gallic acid

Concentration (µg/ml)	Absorbance
1	0.157
2	0.176
4	0.183
6	0.191
8	0.201
10	0.216



Graph 4: Standard Graph of Gallic Acid

IV. DISCUSSION

For alkaloids test, result showed for the extract of *Elettaria Cardamomum*was positive. for ethanolic ethanolic Soxhlet extract solution but absent in maceration extract solution.This showed that *Elettaria Cardamomum*contains nitrogen group which contribute to this reaction.[49]For reducing sugar test, result showed for the extract of *Elettaria Cardamomum*was positivefor both maceration and soxhlet extract solution.[50]For Froth test, result showed for the extract of *Elettaria Cardamomum*was positive. frothing appearance of creamy mist of small bubbles were formed after boiled with distilled water. Hence, saponins were present in the both maceration and soxhlet extract of *Elettaria Cardamomum*. [51]The formations of yellow coloured lower layer indicating the presence of triterpenoids.*Elettaria Cardamomum*contains sterol group.For antraquinones and glycosides test, resultswere negative.For iron (III) chloride test, result was negative. Tannins were not present. The tannins test is to determine the presence of the phenolic compounds but *Elettaria Cardamomum*should have phenolic compound which contribute many therapeutic outcomes. This can be due to the low concentration of the stock dilution and solvent extraction to cause this negative result. Flavonoids were not present in the extract of *Elettaria Cardamomum*. This can be due to the low concentration of the stock dilution and solvent extraction to cause this negative result.For carbohydrate test, result showed for the extract of *Elettaria Cardamomum*was positive [52]. Set 1 and set 2, for antibacterial activity, may spread improperly so that there have irregular zone of inhibition due to improper spreading and diffusion.

- For *Escherichia coli, c*oncentration of 10, 20, 40 mg/ml in set 1, there have no zone of inhibition however in set 2 40mg/ml concentration have zone of inhibition. This may less amount of extract.60mg/ml concentration has the greatest zone of inhibition in set 1. However in set 2 of 60mg/ml the zone of inhibition is small. This may due to different amount of 2 set or may be improper diffusion.
- For *Pseudomonas Aeruginosa, b*oth concentration in set 1 and 2 does not have zone of inhibition except 100mg/ml concentration. This can show that at 100mg/ml is the starting concentration for the antibacterial activity.
- For *Staphylococcus Aureus, i*n both sets, there have zone of inhibition but the zone of inhibition is in irregular shape. This is due to improper spreading and improper diffusion.After 24 hours of incubation, our test tube was observed and chosen the one which is clear. However, there have no any clear solution but the turbidity was increase accordingly. The first tube and the second tube were chosen for each type of bacteria to test the MBC. This is because the first and second test tube took slightly clear. If we increase in the amount of the concentration there may be get a good result with a clear solution. The all agar plate showed the growth of the bacteria on the agar plate. It means that the concentration is not the minimal inhibition concentration for the bacteria growth. In general when our test tube does not show any clear solution in MIC test, no MBC test was conducted.

In DPPH (2,2-diphenyl-1-picryl-hydrazyl) study was used to determine the antioxidant capacity of extract.[53]The evaluation of the antioxidant activity of sample was determined based on the scavenging activity against the free radical DPPH through the calculation of IC_{50}, which represents the concentration of the material required for inhibit 50% of the free radicals. Thus, a lower IC_{50} value in a particular sample indicated a greater ability to neutralize free radical.(54)Both the BHT standard and the plant extract were determined at 518nm. Standard calibration curve was plotted with an equation of y = 0.4046x + 12.03, R^2 = 0.9948 and the *E.Cardamomum* curve was plotted with an equation of y = 0.1265x + 2.2581,R^2 = 0.9311. The absorbance of both BHT standard and Plant extract were recorded in Table 4.6 respectively. It showed that the absorbance

decreases as the concentrations (µg/ml) increase. However, the increase in concentration results in increase of scavenging percentage which results in the linear graph. The results of IC_{50} were calculated based on the standard curve of BHT, the seeds showed an IC_{50} value of 377.41µg/ml compared to the IC_{50} value of standard BHT which is 93.85µg/ml. The plant extract showed higher IC_{50} value than the standard BHT. It indicates seed of *E.Cardamomum* has high activity of to neutralize free radical.[53]The TPC in the leaf extracts using the Folin-Caocalteu's reagent is expressed in terms of Gallic acid equivalent (mg GAE/g). Standard calibration curve was plotted with an equation of $y = 0.0062x + 0.1534$, $R^2 = 0.9825$. The absorbance of each concentration of the extract was recorded. It showed that the absorbance value increase as the concentration (µg/ml) increase. *E.Cardamomum* extract showed total phenolic content of 2.47 mg GAE/gat concentration of 25.0µg/ml. This indicated that seed contains large amount of polyphenol content.

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