Review Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2022.47.007558

Phytochemical Screening, Antibacterial, Total Phenolic Content and Free Radical Scavenging Activity Determination of the Antioxidant Activity Determination of *Syzygium Aromaticum*

Foo Yong Lim[1], Neeraj Paliwal[1], Syed Atif Raza[2], Nabila Perveen[1]* and Naeem Hasan Khan[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, AIMST University, Malaysia*

[2]*University College of Pharmacy, University of the Punjab, Lahore, Pakistan*

***Corresponding author:** Nabila Perveen, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, AIMST University, Malaysia

ARTICLE INFO

Received: December 05, 2022

Published: January 02, 2023

Citation: Foo Yong Lim, Neeraj Paliwal, Syed Atif Raza, Nabila Perveen and Naeem Hasan Khan. Phytochemical Screening, Antibacterial, Total Phenolic Content and Free Radical Scavenging Activity Determination of the Antioxidant Activity Determination of Syzygium Aromaticum. Biomed J Sci & Tech Res 47(5)-2022. BJSTR. MS.ID.007558.

ABSTRACT

Background: To evaluate the phytochemical constituents and antibacterial activity of ethanolic Soxhlet extract.

Method: In the present research, soxhlet extraction was used to extract the *Syzygium Aromaticum* with 95% ethanol as a solvent. Antibacterial activity of the *Syzygium Aromaticum* examined through MIC tests against two ATCC bacterial strains (*Escherichia coli, Bacillus pumilus*).

Result: The ethanolic soxhlet extract of *Syzygium Aromaticum* against Escherichia coli (gram negative) showed negative results, *Bacillus pumillus* (gram positive) showed negative results.

Conclusion: *Syzygium Aromaticum* is a unique spice with many medicinal and traditional uses. It has very wide spectrum of antibacterial activity against many bacterial strains. A long-term research project is a must to evaluate the antibacterial activity with different solvents followed by the zone of inhibition study.

Introduction

Clove, (*Syzygium aromaticum)*, tropical evergreen tree of the family *Myrtaceae* and its small reddish brown flower buds used as a spice. Cloves were important in the earliest spice trade and are believed to be indigenous to the Moluccas, or Spice Islands, of Indonesia. Strong of aroma and hot and pungent in taste, cloves are used to flavour many foods, particularly meats and bakery products; in Europe and the United States the spice is a characteristic flavouring in Christmas holiday fare, such as wassail and mincemeat [1] (Figure 1). As early as 200 BC, envoys from Java to the Han-dynasty court of China brought cloves that were customarily held in the mouth to perfume the breath during audiences with the emperor. During the late Middle Ages, cloves were used in Europe to preserve, flavour, and garnish food. Clove cultivation was almost entirely confined to Indonesia, and in the early 17th century the Dutch eradicated cloves on all islands except Amboina and Ternate in order to create scarcity and sustain high prices [2]. In the latter half of the 18th century the French smuggled cloves from the East Indies to Indian Ocean islands and the New World, breaking the Dutch monopoly. In the early 21st century, Indonesia was the world's largest producer of cloves, followed by Madagascar, Tanzania, and Sri Lanka.

DOI: 10.26717/BJSTR.2022.47.007558

Figure 1: Clove seed (b) clove powder (c-e) clove plant with flowers and raw un-ripe clove seeds.

Botanical Features

The clove tree is an evergreen that grows up to 8–12 metres (26–39 ft.) tall, with large leaves and crimson flowers grouped in terminal clusters. The flower buds initially have a pale hue, gradually turn green, then transition to a bright red when ready for harvest [3]. Cloves are harvested at 1.5–2 centimetres (0.59–0.79) long and consist of a long calyx that terminates in four spreading sepals, and four unopened petals that form a small central ball.

Uses

Cloves are used in the cuisine of Asian, African, Mediterranean, and the Near and Middle East countries, lending flavour to meats,

curries, and marinades, as well as fruit (such as apples, pears, and rhubarb). Cloves may be used to give aromatic and flavour qualities to hot beverages, often combined with other ingredients such as lemon and sugar. They are a common element in spice blends, including pumpkin pie spice and speculaas spices. In Mexican cuisine, cloves are best known as clavos de olor, and often accompany cumin and cinnamon [4]. They are also used in Peruvian cuisine, in a wide variety of dishes such as carapulcra and arroz con leche. A major component of clove taste is imparted by the chemical eugenol, and the quantity of the spice required is typically small. It pairs well with cinnamon, allspice, vanilla, red wine, basil, onion, citrus peel, star anise, and peppercorns.

Non-Culinary Uses

The spice is used in a type of cigarette called kretek in Indonesia. Clove cigarettes were smoked throughout Europe, Asia, and the United States. Clove cigarettes are currently classified in the United States as cigars, the result of a ban of flavored cigarettes in September 2009. Clove essential oil may be used to inhibit mold growth on various types of foods. In addition to these non-culinary uses of clove, it can be used to protect wood in a system for cultural heritage conservation and showed the efficacy of clove essential oil to be higher than boron based wood preservative. Cloves can be used to make a fragrant pomander when combined with an orange. When given as a gift in Victorian England, such a pomander indicated warmth of feeling.

Potential Medicinal Uses and Adverse Effects

Use of clove for any medicinal purpose has not been approved by the US Food and Drug Administration, and its use may cause adverse effects if taken orally by people with liver disease, blood clotting and immune system disorders, or food allergies. Cloves are used in traditional medicine as the essential oil, which is used as an anodyne (analgesic) mainly for dental emergencies and other disorders. There is evidence that clove oil containing eugenol is effective for toothache pain and other types of pain, and one review reported efficacy of eugenol combined with zinc oxide as an analgesic for alveolar osteitis. Clove essential oil may prevent the growth of Enterococcus faecalis bacteria which is often present in a root canal treatment failure. Studies to determine its effectiveness for fever reduction, as a mosquito repellent, and to prevent premature ejaculation have been inconclusive. It remains unproven whether blood sugar levels are reduced by cloves or clove oil. The essential oil may be used in aromatherapy (Table 1).

Table 1: Taxonomical classification of Syzygium aromaticum.

Taxonomy	Classification
Kingdom	*Plantae*
Subkingdom	*Viridiplantae*
Infrakingdom	*Streptophyta*
Superdivision	*Embryophyta*
Division	*Tracheophyta*
Subdivision	*Spermatophyta*
Class	*Magnoliopsida*
Superoder	*Rosanae*
Order	*Myrtales*
Family	*Myrtaceae*
Genus	*Syzygium*
Species	*Syzygium*
	aromaticum

Phytochemical Constituents of Clove

Various studies have been carried out to find various constituents of *S. aromaticum* (SA). Clove buds contain 15–20% essential oil, which is dominated by eugenol (70–85%), eugenyl acetate (15%) and β-caryophyllene (5–12%). Other essential oil ingredients of clove oil are vanillin, crategolic acid, tannins, gallotannic acid, methyl salicylate, flavonoids eugenin, kaempferol, rhamnetin, eugenitin and triterpenoids like oleanolic acid. The constituents of the oil also include methyl amyl ketone, methyl salicylate, α and β-humulene, benzaldehyde, β-ylangene and chavicol. The minor constituents like methyl amyl ketone, methyl salicylate etc., are responsible for the characteristic pleasant odour of cloves. Gopalakrishnan et al. (1984) characterized six sesquiterpenes, α-cubebene (1.3%), α-copaene (0.4%), β-humulene (9.1%), β- caryophyllene (64.5%), γ-cadinene (2.6%) and δ-cadinene (2.6%) in the hydrocarbon fraction of the freshly distilled Indian clove bud oil.

Soxhlet Extraction

Soxhlet extraction is one of the most popular techniques for extraction of analytes from solid materials. Since its discovery in 1879, the standard Soxhlet technique has been routinely applied in almost every analytical laboratory [5]. Up to this day, Soxhlet extraction technique remains a standard technique to which the performance of modern extraction techniques is compared. (Lord, Pawliscyn and Pawliszyn, 2012)

Experimental Methodology

Collection of Plant Material Procedure

500g of Syzygium Aromaticum (SA) were weighed and bought from a local market situated in Sungai petani on 9th April 2022. The initial weight of the clove was taken. The clove was spread on paper and dried using hair dryer. Completely dried clove break into smaller pieces using blender. The weight of the clove pieces recorded again using weighing balance (Figure 2).

Figure 2.

Soxhlet Extraction Method

Typically, Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. The active constituents of the clove were extracted through soxhlet extraction method using ethanol as the solvent [6]. To extract more yield at the same time, 2 soxhlet apparatus were set up by connecting the rubber pipes.

Soxhlet Extraction of Clove Using Ethanol

- The clove was crushed into powder form using a blender and sieved.
- Powdered cloves were weighed using 2 separate weighing boats.
- 300ml of ethanol was measured and transferred into two separate porous extraction thimbles.
- The temperature of the heating mantle adjusted and maintained at 90 degree celsius throughout the extraction process.
- The soxhlet extraction took about 12 hours to complete the extraction process. The end point of the extraction process is determined by observing decolorization of brown colour clove powders to colourless form.
- The soxhlet extract was collected from both round bottom flask and filtered into a 1000ml beaker with muslin cloth to avoid any smaller clove from the powder to remain in the extract.
- The mouth of the beaker containing soxhlet extract completely covered with aluminium foil and labeled accordingly.

Evaporation of Extract Using Rotary Evaporator

1. Soxhlet extract in the beaker transferred into the specified flask of the rotary evaporator accordingly.
2. The temperature of the heating water bath was set up to 65 degree celsius and the speed of the rotator set to 100 rpm. This evaporation process required approximately 8 hours to be completed.
3. The extract was measured and collected in a conical flask in the end of the process. The recovered ethanol obtained from this process also collected.
4. The final volume of soxhlet product collected from this process was 125ml.
5. The soxhlet extract was transferred into respective beakers. The mouth opening of beaker were covered with aluminium foil to prevent the evaporation.

Concentration of Extract Using Water Bath

1. Two porcelain dishes were weighed and the weight recorded.

2. The evaporated soxhlet extract was measured using measuring cylinder and were transferred to two porcelain evaporating dishes.
3. The dishes were covered with aluminium foil and several holes were made on the aluminium foil to allow the evaporation of vapor from the extract.
4. The two porcelain evaporating dishes were placed on Digital Heating Thermostatic temperature-constant Water Bath.
5. The temperature of the water bath was set to 65 degree celsius and the water level of water bath was made sure to reach minimum level in order to prevent any exhausting of water which will cause damage to the equipment.
6. The evaporation of both extracts took place for 5 days.
7. The liquid form of extract turned into semi-solid form or solid form or gummy form in the end of the process.
8. The net weight of soxhlet extract after evaporation was 25.83g net.
9. The dishes were covered with aluminium foil and were stored in the freezer and kept for further use in determination of antibacterial activity (Figure 3).

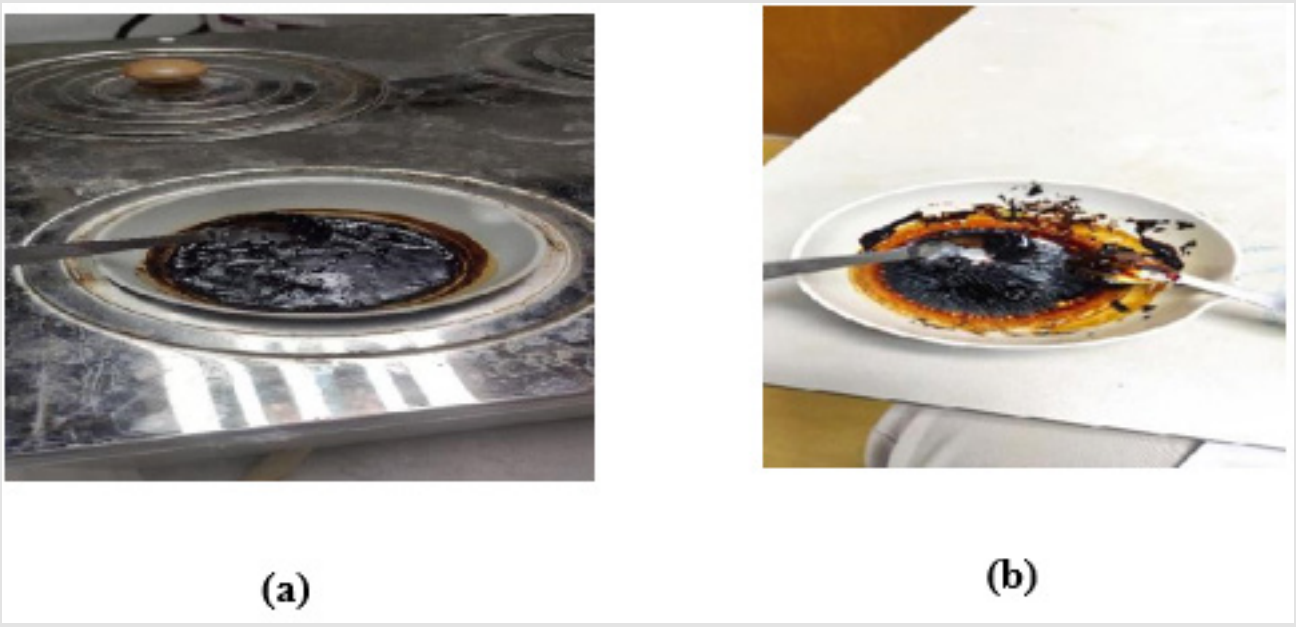


Figure 3:
a. Soxhlet extract during evaporation
b. End product of soxhlet extract in solid form.

Antibacterial Activity of Plant Extract

Preparation of Muller-Hinton Agar, Muller-Hinton Broth and Autoclave

800 ml of MHA was prepared by weighing 30.48 gram of MHA powder and mixed in two screwed-cap bottles with 400 ml each and stirred using a glass rod to ensure proper mixing. Next, 260 ml 0f MHB was prepared using the instruction on bottle behind. The prepared MHB were inserted into 24 test tubes, 9ml each using micropipette, and covered with cotton wool and aluminum foil. They were autoclaved Three universal bottles filled with distilled water were also autoclaved.

Preparation of MHA Plate Using Pour Plate Technique

33 sterilized empty petri plates were obtained from the lab assistant. After autoclave, the prepared MHA is poured into each petri plate until they were half-filled. This procedure was carried out in a fume hood to prevent contamination [7] MHA were allowed to dry for 20 minutes and stored into the fridge for further use (Figure 4).

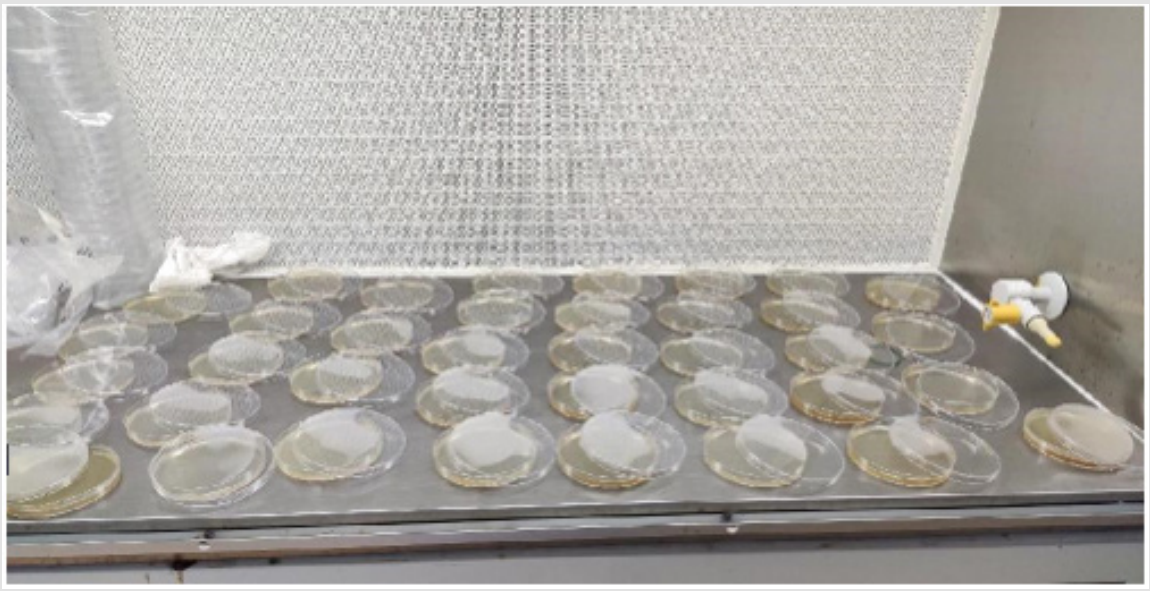


Figure 4: MHA plate.

Subculture of *Bacillus Pumilus* and *E. Coli* from Mother Culture

The mother culture broth of *Bacillus pumilus* and *E. coli* were provided by lab assistant. The two conical flasks contain 25 ml of nutrient broth the two bacteria strain were labelled. The mother culture was mixed by swirling the conical flask gently. The loop was sterilized by dipping into ethanol 70% and flamed. A loop of culture was transferred from the mother culture into the sterile broth and mixed well [8]. The flame was applied immediately to sterilize the inoculating loop after the transfer. The conical of newly transferred *Bacillus pumilus* and *E. coli* were incubated at 37°c for 24 hours. The growth of bacteria broth culture was observed [8].

Preparation of Macfarland Standards

A universal bottle was prepared. 0.1 g of Barium chloride was weighed and mixed in 10 ml of distilled water to produce 1% Barium chloride. Next, 1% sulphuric acid was prepared by mixing 10ul of concentrated sulfuric acid in 9.99ml of distilled water. A 0.5 MacFarland standard was prepared by mixing 0.05ml of 1% Barium chloride with 9.95ml of 1% sulfuric acid and poured into the universal bottle [9].

Preparation of Plant Extract Stock Solution

4mg/ml of plant extract stock solution was prepared by weighing 80mg of plant extract and mixed with 20ml of 5% ethanol. The mixture was sonicated to ensure proper mixing. The stock solution is then poured into a universal bottle and stored [10] (Figure 5).

Figure 5: Plant extract solution.

Preparation of Penicillin Stock Solution

10mg/ml concentration of penicillin stock solution was prepared and stored in a 10ml centrifuge tube [11].

Comparison of Macfarland Standard with Bacterial Culture

Each of the bacterial culture was compared with MacFarland standard by adding nutrient broth with incubated bacteria drop by drop into the autoclaved universal bottle with distilled water until their turbidity becomes equivalent to see the equal concentration with the help of Turbidity Comparison Card.

Broth Dilution for Mic Test

The first test tube was filled with 5ml of MHB and the remaining 6 test tubes were filled 9ml of MHB. The test tubes were labeled with number of repetition, bacterial strain involved and concentration of broth dilution (0.0625mg/ml, 0,125mg/ml, 0.25mg/ml, 0.5mg/ml, 1.0mg/ml, 2.0mg/ml and 4.0mg/ml). Another 3 test tubes were filled with 9ml of MHB with the label: Bacteria + penicillin (B+A), Bacteria only (B) and MHB only (N/E). Next, 4ml of plant extract was added into the first test tube contain 5ml of MHB using a micropipette. 5ml of the solution was transferred from the first test tube to the second test tube and re-suspended using a micropipette. After that, another 5ml of solution from the second test tube was transferred to the third test tube and re-suspended. This step was repeated until the 7th test tube. 1ml of bacterial culture was added into each test tube except the three positive control test tube [12]. After the serial dilution, the test tubes were incubated in a bacteriological incubator at 37oc for 24 hours. The MIC result were observed and determined.

Incubation of Sample Using Spread Plate Technique

A dilution series was made from the sample. 100ul was pipette out from the each of the dilution series onto the center of the surface of an agar plate. The L-shaped glass spreader was spread with 70% alcohol and flamed over a Bunsen burner for sterilization. The sample was spread evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at the same time. The plate is then incubated at 37°c for 24 hours [13].

Observation and Interpretation of Result

After incubation of 24 hours, the plates were observed for viable plate count, in which the total number of colonies forming unit on a single plate is enumerated. The lowest concentration of each extract displaying no visible growth was recorded as the minimum inhibitory concentration. The concentration that inhibited bacterial/yeast growth completely (the first clear well) was taken as the MIC value. MIC values were determined in triplicate and repeated to confirm activity

Antibacterial Activity of *Syzygium Aromaticum* on *E. Coli*

Three repetitions (A1-A7, B1-B7, C1-C7) have been done on each bacterial strain against ethanol plant extract

Result

N/E = Plain agar

E.Coli (Figure 6).

DOI: 10.26717/BJSTR.2022.47.007558

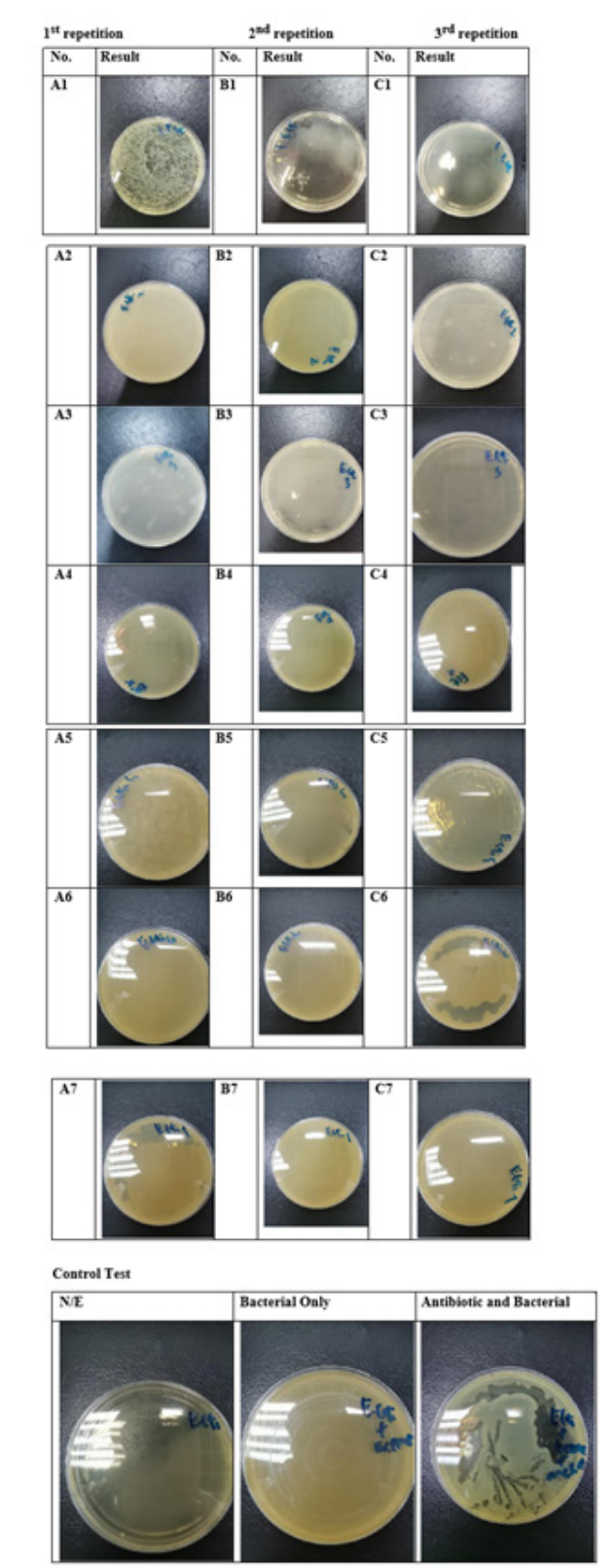


Figure 6: Antibacterial activity observation and interpretation of agar plate against *E.coli*.

 DOI: 10.26717/BJSTR.2022.47.007558

Interpretation

Ethanol extract of Syzygium Aromaticum does not showed any antibacterial activity against *E. coli* since there is no observation of MIC.

Antibacterial Activity of *Syzygium Aromaticum* on *Bacillus Pumilus*

Three repetitions (A1-A7, B1-B7, C1-C7) have been done on each bacterial strain against ethanol plant extract.

Result

N/E = Plain agar (Figure 7).

Bacillus pumilus.

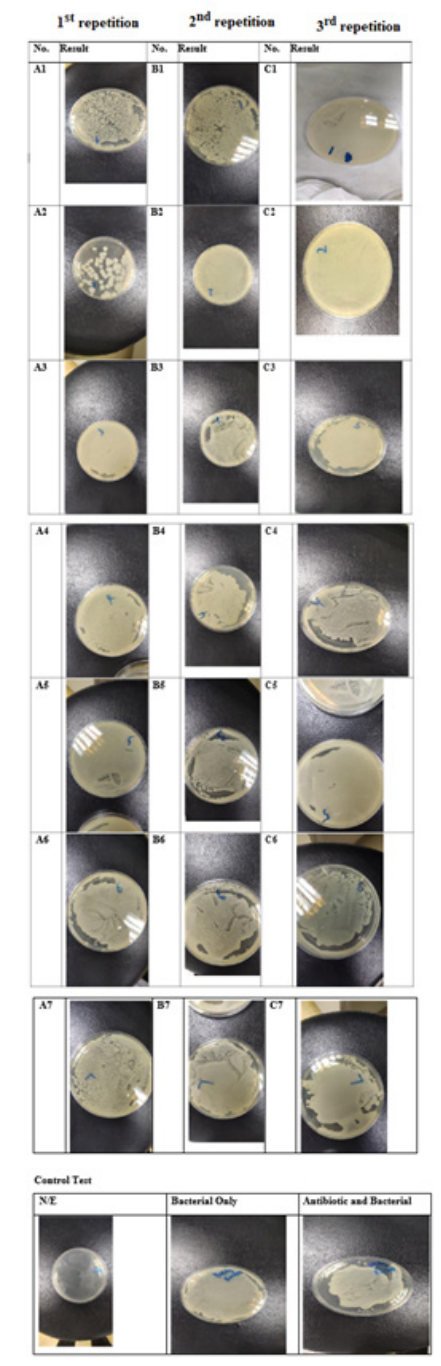


Figure 7: Antibacterial activity observation and interpretation of agar plate against *Bacillus pumilus*.

Interpretation

Ethanol extract of Syzygium Aromaticum does not showed any antibacterial activity against *Bacillus pumilus* since there is no observation of MIC.

Test for Free-Radical Scavenging

Activity DPPH Assay

DPPH is 2, 2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$) to determine the antioxidant activity of the tested compound. In this test, the scavenging capacity of antioxidant towards DPPH was measured. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidant to the corresponding hydrazine. Therefore, DPPH assay is actually related to the hydrogen donating ability of the tested extract. DPPH will produce violet colour in methanol solution but fades to shades of yellow colour upon scavenged. The procedure for conducting DPPH assay was mentioned below.

Preparation of 1,1-Diphenyl-2-Picryl Hydrazyl (Dpph) Solution

0.3 mM of DPPH reagent was prepared by dissolving 11.83 mg in 100 ml of ethanol.

DPPH Assay Procedure

2.5 ml of different concentrations of maceration extract of SA were added into 6 separate test tubes. All the test tubes were then covered with aluminium foil as DPPH is a photosensitive reagent. After that, 1 ml of 0.3 mM of alcoholic reagent of DPPH was added into the test tubes. The test was performed in triplicate for each concentration of maceration extract of SA. The procedure mentioned above was repeated with the Soxhlet extract of SA and ascorbic acid solution. Similarly, control solution was prepared by replacing the extract with ethanol and addition of 1ml of 0.3 mM of alcoholic solution of DPPH. A blank solution was prepared only with 3ml of 95% ethanol. All the test tubes containing the preparation mentioned above were allowed to stand for 30 minutes in dark cupboard. The absorbance at 518nm of different preparations were studied by using UV-Visible spectrophotometer. The test needed to be performed in a dark condition. The absorbance values of each different concentration of standard ascorbic acid, SA extract, control and blank were recorded. The free radical scavenging activity of different concentration of SA extract was calculated by using the formulae given below [4,14].

$$DPPH\ Radical\ Scavenging\ Activity\ (\%) = (A_{control} - A_{sample}) / A_{control} \times 100\%$$

$A_{control}$ =absorbance of controls

A_{sample} = absorbance of the sample extracts

Test for Total Phenolic Content

Folin-Ciocalteu Method

The total phenolic content in ethanol extract of SA was determined through Folin-Ciocalteu method. Folin-Ciocalteu reaction is an antioxidant assay based on electron transfer, which measure the reductive capacity of tested compound. It is widely applied in the determination of total phenolic content in the sample as the phenolic content is closely related to the antioxidant activity of a particular compound. The total phenolic of the extract is then calculated based on standard curve prepared using Gallic acid and expressed as mg of Gallic acid equivalent (GAE)/g of dry extract. The total phenolic content in extract is expressed in term of Gallic acid equivalent [4].

Preparation of Plant Extract and Gallic Acid Solution

Stock solution of 1 mg/ml was prepared by dissolving 10 mg of SA extract obtained from maceration process in 10 ml of methanol. This step was repeated by using the SA extract obtained from the Soxhlet extraction. Only 1 concentration of SA extract was needed in this test. A stock solution of Gallic acid was prepared by dissolving 100 mg of Gallic acid in 100 ml of methanol. Serial dilution method was then performed in order to obtain 6 different concentrations of Gallic acid solution. From the freshly prepared stock solution of Gallic acid, 1.0, 0.8, 0.6, 0.4, 0.2, 0.1 ml were pipetted out and made up to 10 ml by methanol individually to produce 10, 20, 40, 60, 80, 100 µg/ml respectively.

Total Phenolic Content Test

2.5 % sodium carbonate solution was prepared by dissolving 2.5 g of sodium carbonate in 100 ml of distilled water. One ml of stock solution of SA was added into a test tube by using a micropipette. The test tube containing the stock solution of SA was added with 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5 % sodium carbonate solution. The steps above were repeated in triplicate for each SA stock solution obtained from different extraction method. Different concentration of Gallic acid solution was added into separate, labelled test tubes. 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5 % sodium carbonate solution were also added into the test tube containing the Gallic acid solution. Similarly, a control solution was prepared by replacing the SA extract with 1 ml of methanol and addition of 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5 % sodium carbonate solution. Besides, a blank solution was prepared by using only 3 ml of methanol. All the test tubes containing different concentrations of standard Gallic acid, SA extract, control as well as blank were allowed to stand for two hours. Next, all the test tube containing different concentrations of standard Gallic acid, SA extract, control and blank were subjected to UV visible analysis for

the absorbance at 760 nm by using UV-Visible spectrometer . The absorbance value of each different concentrations of standard Gallic acid, SA extract, control and blank were then recorded as shown in Tables 2-4. The Table 5 shows the result of total phenolic content. Standard curve of Gallic acid is shown in Graph 1. Table 2.

Table 2.

Concentration of SA maceration extract		Absorbance at 518nm	DPPD Radical Scavenging Activity (%) (Acontrol –Atest) / Acontrol ×100 %
100 µg/mL		0.582	52.70%
80 µg/mL		0.63	48.80%
60 µg/mL		0.659	46.40%
40 µg/mL		0.718	41.60%
20 µg/mL		0.735	40.20%
10 µg/mL		0.783	36.3
Control	1.23	-	

Table 3.

Concentration of SA soxhlet extract	Absorbance at 518nm	DPPD Radical Scavenging Activity (%) $(A_{control} - A_{test}) / A_{control} \times 100$ %
100 µg/mL	0.101	50.00%
80 µg/mL	0.12	40.60%
60 µg/mL	0.131	35.10%
40 µg/mL	0.14	30.70%
20 µg/mL	0.163	19.30%
10 µg/mL	0.182	9.90%
Control	0.202	-

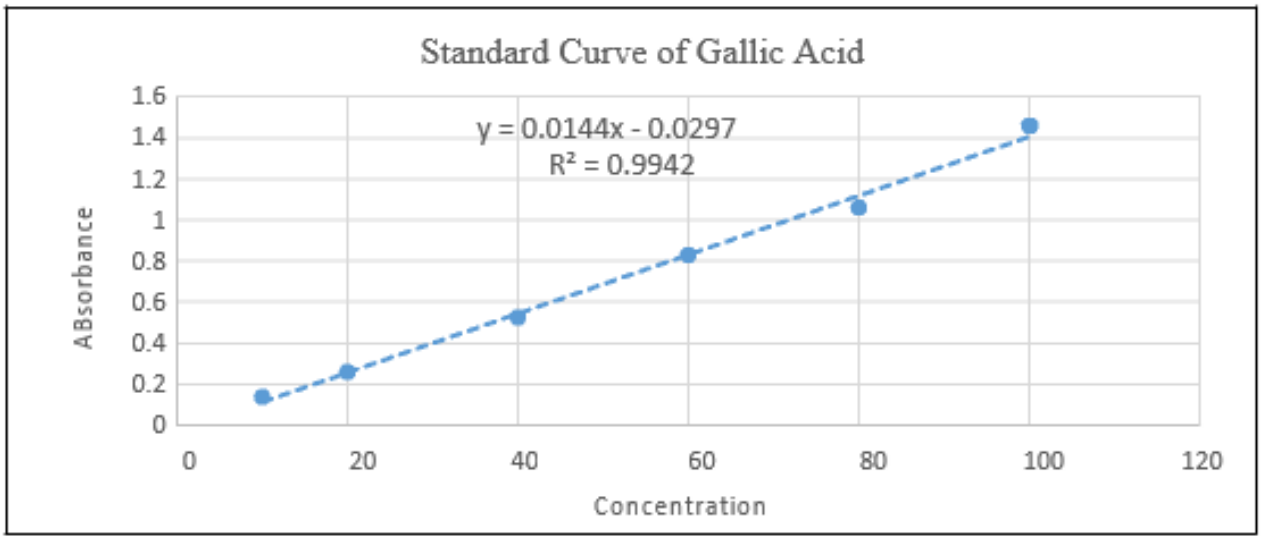
Table 4.

Concentration of ascorbic acid		Absorbance at 518nm	DPPH Radical Scavenging Activity (%) $(A_{control} - A_{test}) / A_{control} \times 100$ %
100 µg/mL		0.026	87.10%
80 µg/mL		0.025	87.60%
60 µg/mL		0.024	88.10%
40 µg/mL		0.026	87.10%
20 µg/mL		0.023	88.60%
10 µg/mL		0.09	55.40%
Control	0.202	-	

Table 5: Result of Total Phenolic Content.

Sample	Absorbance at 760 nm
SA maceration extract (1mg/ml)	0.269
SA soxhlet extract (1mg/ml)	0.366
Gallic acid 100 µg/mL	1.462
Gallic acid 80 µg/mL	1.061
Gallic acid 60 µg/mL	0.831
Gallic acid 40µg/mL	0.527

Gallic acid 20µg/mL	0.261
Gallic acid 10 µg/mL	0.138
Control	0.019
Blank	0.003



Graph 1: Standard curve of Gallic acid.

Calculation of Total Phenolic Content in C.Asiatica Extract

Given,

y = 0.0144x - 0.0297, where x=concentration of phenolic compound, y = absorbance value Maceration extract, absorbance =0.269,

Concentration of phenolic compound = (0.269+0.0297) /0.0144

=20.74 µg/ml Gallic acid equivalent

Soxhlet extract, absorbance = 0.366

Concentration of phenolic compound = (0.366+0.0297)/0.0144

= 27.48 µg/mL Gallic acid equivalent

Phytochemical Analysis and Discussion

Ethanol Extract of Syzygium Aromaticum

The aqueous extract of Syzygium Aromaticum was carried out by hot extraction method. Soxhlet extractor are simple and clear design, production process continuity, ease of visual monitoring of the process, a low flow of solvent and the possibility of its reuse after stripping and distillation. Soxhlet extraction is a simple and effective method. Soxhlet extraction has traditionally been used for a solid sample with limited solubility in a solvent in the presence of insoluble impurities. A porous thimble loaded with a solid sample is placed inside the main chamber of the Soxhlet extractor [15] By refluxing the solvent through the thimble using a condenser and a siphon side arm, the extraction cycle is typically repeated many times. Soxhlet extraction is a rugged, well-established technique and permits unattended extraction. However, it requires a long extraction time and the consumption of a large amount of solvent. The use of nonpolar solvents only is not recommended. In this research work, ethanol is used as the solvent. Most importantly ethanol is both highly effective and perfectly safe to use for plant extraction [16]. Ethanol will retrieve a large amount of oil from the plant and then completely evaporate. This results in the maximum amount of product from plant that is safe to be used. Ethanol allows extraction of both water soluble and oil soluble components [17]. Phytochemical results are given in Table 6.

Table 6: Qualitative analysis of the phytochemicals of ethanol extract of *syzygium aromaticum* plant.

Phytochemical results		
S.NO.	**PHYTOCHEMICAL TEST**	**RESULT**
1	Test for Carbohydrates Molisch's Test	-
2	Test for Monosaccharides Barfoed's Test	+
3	Test for Polysaccharides Iodine Test	+
4	Test for Reducing Sugars Fehling's Test Benedict's Test	+ +
5	Test for Pentose Sugar Bial's Test	-
6	Test for Hexose Sugar Seliwanoff's Test Tollen's Phloroglucinol test	- +
7	Test for Protein Xanthoprotein Test Million Test	+ -
8	Test for Amino Acid Ninhydrin Test Lead Acetate Test Nitroprusside Test	- + +
9	Test fof Lipids and Fatty Acids Test for Free Fatty Acid Copper Acetate Test	- +
10	Test for Volatile Oil Filter Paper Test	-
11	Test for Terpenoids or Steroids Liebermann-Burchard Salkowski Test	- -
12	Test for Glycosides Test for Glycosides	+
13	Test for Anthraquinones Glycosides Modified Borntrager's Test	-
14	Test for Cardiac Glycosides Keller-Killiani's Test	-
15	Test for Cyanogenic Glycosides Ferriferrocyanide Test	-
16	Test for Coumarin Glycosides Ferric Chloride Test	+
17	Test for Saponins Glycosides Foam Test	+

18	Test for Alkaloids Test Dragendorff's Test Wagner's Test Hager's Test	 + + +
19	Test for Flavonoids Test with Sodium Hydroxide Solution Test with Lead Acetate Solution Shinoda Test	 - - -
20	Test for Phenols Liebermann's Test Test with dilute potassium permanganate solution	 - -
21	Test for Tannins Ferric Chloride Test Bromine water Test	 + -
+: Presence	-: Absence	

Conclusion

In the present research, phytochemical screening and evaluation of antibacterial activity by using ethanol extract of *syzygium aromaticum* plants were successfully carried out. The phytochemical screening carried out on ethanol extract of clove showed the presence of carbohydrates, monosaccharides, polysaccharides [18-22], reducing sugars, hexose sugars, protein, amino acids, saturated fatty acids, glycosides, Coumarin Glycosides, Saponins Glycosides, Alkaloids and tannins. On the other hands, the extract showed absence of pentose sugars, volatile oils, terpenoids and steroids, Anthraquinones Glycosides, Cardiac Glycosides, Cyanogenic Glycosides, flavonoids as well as phenol. The antibacterial activity of clove extract was conducted through Minimum Inhibitory Concentration (MIC) tests using broth dilution method to determine the antibacterial properties [23]. The study was carried out against three bacterial strains including *Bacillus pumilus* and *Escherichia coli*, antibacterial activity of clove was not observed against the two strains [24]. However more strains can be used to test for antibacterial activity since it possesses many kinds of phytochemicals.

Funding Details

The authors are highly thankful to the Faculty of Pharmacy, AIMST University, Bedong, Kedah D.A., Malaysia for funding and providing facilities to carry out this research project.

Acknowledgement

Thanks to science officers Miss Amalina and Miss Jaya.

Conflict of Interest

Authors declare that there is no conflict of interests.

References

1. Cortés-Rojas D, de Souza C, Oliveira W (2014) Clove (Syzygium aromaticum): a precious spice. Asian Pacific Journal of Tropical Biomedicine 4(2): 90-96.
2. Gosavi N, Koli S, Jire D, Shaikh A (2020) Clove (Syzygium Aromaticum): A Miraculous Spice. American Journal of PharmTech Research 8(5): 1-17.
3. Syzygium aromaticum (L) Merr & L M Perry (2022) Plants of the World Online. Kew Science. Plants of the World Online.
4. Zhao C, Liang Y, Fang H, Li X (2005) Temperature-programmed retention indices for gas chromatography–mass spectroscopy analysis of plant essential oils. Journal of Chromatography A 1096(1-2): 76-85.
5. Zygler A, Słomińska M, Namieśnik J (2012) Soxhlet Extraction and New Developments Such as Soxtec. Comprehensive Sampling and Sample Preparation, p. 65-82.
6. Singh V (2022) Soxhlet Extraction - Principle, Working, Uses with Diagrams. PharmaGroww.
7. Aryal S, Hardeen T, Varquez F, zeb Z, Bh S (2022) Mueller Hinton Agar (MHA) – Composition, Principle, Uses and Preparation. Microbiology Info.com.
8. Choudhary A (2022) SOP for Procurement, Maintenance and Subculturing of Standard Microbial Cultures. Pharmaguideline.com.
9. Aryal S (2022) McFarland Standards- Principle, Preparation, Uses, Limitations. Microbe Notes.
10. Abubakar A, Haque M (2020) Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy And Bioallied Sciences 12(1): 1.
11. (2022) Stock Solution. Microbiology and Molecular Biology.
12. (2003) Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. Clinical Microbiology and Infection 9(8): ix-xv.
13. Aryal S (2022) Spread Plate Technique- Principle, Procedure and Uses. Microbiology Info.com.
14. Fu Y, Zu Y, Chen L, Shi X, Wang Z, et al. (2007) Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytotherapy Research 21(10): 989-994.

15. (2022) Molisch's Test - Principle, Procedure, Reaction, & Reagent Preparation. BYJUS.

16. (2022) Soxhlet Extractor. Chembam.com.

17. Haro-González J, Castillo-Herrera G, Martínez-Velázquez M, Espinosa-Andrews H (2021) Clove Essential Oil (Syzygium aromaticum L. Myrtaceae): Extraction, Chemical Composition, Food Applications, and Essential Bioactivity for Human Health. Molecules 26(21): 6387.

18. Gülçin İ (2011) Antioxidant Activity of Eugenol: A Structure–Activity Relationship Study. Journal of Medicinal Food 14(9): 975-985.

19. JE O, JO S, EE U (2022) Hepatoprotective activity of the extract of Homalium letestui stem against paracetamol-induced liver injury. PubMed.

20. Pulikottil S, Nath S (2022) Potential of clove of Syzygium aromaticum in development of a therapeutic agent for periodontal disease: A review. Scielo.org.za.

21. Khan R, Islam B, Akram M, Shakil S, Ahmad A, et al. (2009) Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Molecules 14(2): 586-597.

22. (2022) Ethanol Grades & Purity for Extraction. Production Automation Station.

23. Sapkota A (2022) Barfoed's Test- Definition, Principle, Procedure, Result, Uses. Microbe Notes.

24. Sapkota A (2022) Nitroprusside Test- Definition, Principle, Procedure, Result, Uses. Microbe Notes.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2022.47.007558

Nabila Perveen. Biomed J Sci & Tech Res

Submission Link: https://biomedres.us/submit-manuscript.php