ORIGINAL ARTICLE

Antibacterial and Antioxidant Activities of Ghee Hiang Sesame Oil Extract

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ABSTRACT

Introduction: Sesame (*Sesamum indicum*) is an oil-producing plant, with seeds that contain 50 to 60% oil and 25% protein. Sesame oil is widely used as a seasoning in Asian cuisine due to its flavour and aroma. It contains a high concentration of bioactive compounds, particularly lignans, vitamin E, and phytosterols. Thus, this study was aimed to evaluate antibacterial and antioxidant activities of Ghee Hiang sesame oil extract. **Methods:** The sesame oil was provided by Ghee Hiang Manufacturing Co., Penang, Malaysia. The sesame oil was then extracted with methanol using liquid partitioning method. The antibacterial activity of the sesame oil extract was determined on disc diffusion and broth microdilution assays. Then, the antioxidant activity of the extract was determined using diphenylpicryl-hydrazyl (DPPH) radicals. **Results:** Out of 8 test microorganisms, 4 Gram positive bacteria and 2 Gram negative bacteria were susceptible to the extract. The antibacterial activity was broad spectrum. Minimal inhibitory concentrations (MIC) of the sesame oil extract ranged from 3.1 to 12.5 mg/mL, where the minimal bactericidal concentrations (MBC) ranged from 6.3 to 25.0 mg/mL. The MBCs were significantly higher than MIC. DPPH scavenging activity of sesame oil extract was concentration dependent. The sesame oil extract at 1000 µg/mL showed the highest antioxidant activity, and an IC50 of 120.9 µg/mL was recorded. **Conclusion:** Ghee Hiang sesame oil extract showed significant antibacterial and antioxidant activities. Further investigations should be done to determine the bioactive entities present in the extract.
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Keywords: *Sesamum indicum*; Sesame oil; Antibacterial activity; Antioxidant

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INTRODUCTION

Sesame oil is derived from *Sesamum indicum* plant. Sesame plant is originated from Africa (1). It is also known as gingelly, til, benne seed, and the "Queen of Oilseeds" due to its high resistance to oxidation and rancidity (1). *S. indicum* has been domesticated for well over 5000 years. The seeds, seed oil, and various plant parts have been used to cure a variety of illnesses or disorders, including ulcers, asthma, amenorrhea, haemorrhoids, wound healing and inflammations (2). Sesame oil is a fragrant oil derived from sesame seeds, which is a traditional product made from sesame seeds (3). Sesame oils are widely used in Asian cooking and bread pastries as a flavouring agent. Besides, sesame oil is also used in the chemical industry to produce margarine, cosmetics, perfumes (1). Sesame oil showed antibacterial properties on *Verruca vulgaris* and *Verruca plana*, which often present on the foot's soles and around the toes (4). Besides, sesame peptides with molecular mass of less than 1 kDa also inhibits the growth of Pseudomonas aeruginosa (5).

Sesame oil contains a high concentration of bioactive substances such as lignans, vitamin E, and phytosterols (1). The cold-pressed sesame produces oil with high quality and good nutritional content. Linoleic acid (46.9%) is the most abundant unsaturated fatty acid in sesame oil which can

help prevent several diseases including heart disease and cancer (6). These fatty acids are considered essential because they cannot be synthesised in the body. Furthermore, sesame oil is high in vitamin E, the majority of which is gamma-tocopherol (90.5%) (6). Sesame oil has a high oxidative stability due to the presence of natural antioxidants such as sesamol, sesamolin, and gamma-tocopherol, which prevents oxidation and free radical attack (3). Sesame seed is rich 50-60% polyunsaturated fatty acids with great antioxidant activity (2). These bioactive components are essential for improving sesame oil's stability and shelf life while also delivering several health advantages to the consumers (7).

Sesame seeds are rich in protein, dietary fibre, phosphorous, vitamin B1, manganese, copper ,iron, magnesium, calcium, and zinc (4). Besides, sesame oil also showed significant analgesic, antipyretic and anti-inflammatory effects in animal models (8). In 2020, Malaysia shipped a total of 2,053 tonnes of sesame oil. Malaysia exported 1,481 tonnes of sesame oil in 2019 (9). Compared to 2018, the demand for Malaysian sesame oil has increased by 13.836% in 2019. Between 2017 and 2019, exports of sesame oil increased by 28.67%, earning the exporter $4.13 million in 2019 (9). Thus, this study was aimed to evaluate the antibacterial and antioxidant activities of the methanolic extract obtained from Ghee Hiang sesame oil, which is manufactured in Malaysia.

MATERIALS AND METHODS

Extraction of sesame oil

The sesame oil was provided by Ghee Hiang Manufacturing Co., Penang, Malaysia. The sesame oil was then extracted with methanol using liquid partitioning method (10). A volume of 100 mL of each sesame oil was extracted with 100 mL of methanol, at a ratio of 1:1 (V/V) in a separation funnel. The upper organic layer was collected as the methanolic extract of sesame oil. The extracts were evaporated using a rotary evaporator (IKA) at room temperature (28℃). After that, the concentrated extract was dried at 50℃ for 48 hours. The extraction yield was calculated based on the formula below:

$$\text{Extraction yield (\%)} = \frac{\text{The weight of extract}}{\text{The weight of sample}} \times 100$$

The extract was dissolved with methanol to the desired concentration for antibacterial and antioxidant assays. The extract was sterilized by using PTFE membrane filter with 0.2 µm pore size (Whatman).

Test bacteria

Four of Gram positive bacteria namely *Streptococcus pneumoniae* ATCC49619, *Bacillus subtilis* ATCC6051, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, *S. aureus* ATCC29213 and 4 Gram negative bacteria, *P. aeruginosa* PAO1, *Klebsiella pneumoniae* ATCC13883, *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922 were used. The bacterial cultures were maintained at Universiti Kuala Lumpur, Institute of Medical Science Technology (UniKL MESTECH). The bacteria were sub-cultured on nutrient agar (Oxoid) slants and stored in refrigerator until required (4℃). Bacterial suspension for antibacterial assays was prepared by transferring few colonies of 24 hours-old bacterial cultures into 5 mL of sterile physiological saline. The suspension's turbidity was adjusted to meet the 0.5 McFarland standard. The bacterial suspension had a density of about 1.5 X 10^8 CFU/ml.

Disc diffusion assay

Mueller Hinton agar (MHA) (Oxoid) was used for this assay (11). The sesame oil extract was made at a concentration of 100 mg/ml by dissolving 100 mg of the crude extract paste in 1 ml of methanol. With a sterile cotton swab, the bacterial inoculum was streaked onto the agar plate. On top of the inoculation media, a sterile paper disc (6 mm in diameter) impregnated with 20 µL of sesame oil extract was placed. Thereafter, 20 µL of 25 µg/ml Chloramphenicol (Merck) and 20 µL of methanol were used as positive and negative controls, respectively. The plates were incubated for 24 hours at 37°C. Using a ruler, the diameters of the clear inhibition zones surrounding the paper discs were measured after incubation. The experiment was conducted three times, and the diameter of the clear zone was given as the mean diameter ± standard deviation.

Broth microdilution assay

The test bacteria that showed significant inhibitory activity on disc diffusion assay were selected for this assay (11). The sesame oil extract at various concentrations were prepared by performing serial double dilution with methanol, the concentrations were 100.0, 50.0, 25.0, 12.5, 6.3, 3.1, 1.6, and 0.8 mg/ml. After that, 100 µL of each bacteria inoculum was added with 900 µL of double strength Mueller Hinton broth (Oxoid) was added. The assay was conducted in a sterile 96-well plate with a flat bottom (Nest). In order to attain a final volume of 200 µL in each well, 100 µL of extract and 100 µL of bacterial suspension were added. The final concentration of sesame oil extract ranged between 0.4 and 50.0 mg/ml. The sterility control was prepared by combining one for each extract concentration with sterile double strength Mueller Hinton broth (Oxoid). The growth control was then implemented by introducing methanol to the bacterial inoculum, and the final concentration of methanol was 50%. After 24 hours of incubation at 37°C, the minimal inhibitory concentration (MIC)

was determined by adding 40 µL of 0.4 mg/ml iodotetrazolium violet (INT) salt solution in ethanol to detect the growth of bacteria. For the determination of the minimal bactericidal concentration (MBC), each well's sample was streaked on a Mueller Hinton agar plate. The vitality of the test microorganisms was assessed after 24 hours of incubation at 37°C by detecting the presence of bacterial colonies. The minimal bactericidal concentration was defined to be the lowest concentration of extract capable of killing the test bacteria.

Antioxidant activity

The scavenging activity of the extract for diphenylpicryl-hydrazyl (DPPH) radicals was tested (12). The DPPH stock solution (Sigma, USA) was prepared by dissolving in 100% methanol, to achieve a concentration of 0.16 mM based on the formula below.

$$\text{Amount of DPPH used (g)} = \frac{\text{Molecular weight of DPPH x Concentration (M) x Volume (mL)}}{1000}$$

In this study, α-Tocopherol (Sigma, USA) was used as positive control. To achieve a final concentration of the extract ranging from 0 to 1000 µg/mL, 100 µL of the extract was added to 100 µL of freshly prepared DPPH solution. Owing to the colour of the extract, 200 µL of a blank sample containing no DPPH solution was added. The 96-well plate (Nest) was incubated for 30 minutes in the dark at room temperature (25°C). The absorbance of the mixture was measured at 513 nm using a Thermo Multiskan microplate reader following the incubation period (Thermo Fisher, USA). Asample represents the absorbance of the test sample, Ablank represents the absorbance of the sample without DPPH solution, and the control represents the absorbance of the negative control (without extract).

$$\text{Percentage of scavenging activity (\%)} = \frac{1 - (A_{sample} - A_{blank})}{A_{control}}$$

A graph of extract concentration versus DPPH scavenging activity was plotted. Using linear regression analysis, the IC50 values were calculated to show antioxidant activity of the extract.

RESULTS

Extraction of sesame oil

Liquid partitioning was performed to extract the sesame oil sample. In a separation funnel, 2 distinct layers were formed, the upper layer was light brown in colour, and the bottom layer was dark brown in colour. The upper organic layer was collected as methanolic extract of the sesame oil. After drying, the extract appeared was brown in colour and extraction yield of 1.74% was obtained.

Disc diffusion assay

On disc diffusion assay, out of 8 test microorganisms, 4 Gram positive bacteria and 2 Gram negative bacteria were susceptible to the extract (Table I).

Table I : The antibacterial activity of sesame oil extract on disc diffusion assay

Test Bacteria	Mean Diameter of Inhibition Zone (mm)		
	Sesame oil extract (100 mg/ml)	Chloramphenicol (Positive Control)	Methanol (Negative Control)
Gram Positive Bacteria			
Streptococcus pneumoniae ATCC49619	9.5±0.9	34.0±2.1	-
Bacillus subtilis ATCC6051	10.5±1.1	24.0±1.9	-
MRSA ATCC43300	12.5±1.2	35.5±2.4	-
Staphylococcus aureus ATCC29213	13.5±1.1	34.0±1.8	-
Gram Negative Bacteria			
Pseudomonas aeruginosa PAO1	-	17.5±1.3	-
Klebsiella pneumoniae ATCC13883	-	30.0±2.1	-
Escherichia coli ATCC 8739	9.5±1.0	28.0±1.4	-
Escherichia coli ATCC 25922	10.5±1.2	28.5±1.6	-

(-) = No inhibitory activity

The antibacterial activity of the sesame oil extract was broad spectrum, as it inhibited both Gram positive and Gram negative bacteria. For sesame oil extract, the zone sizes were ranged from 9.5 to 13.5 mm. The biggest inhibition zone was recorded on a Gram positive bacterium, *S. aureus* ATCC29213 with a diameter of 13.5 mm (Figure 1). The smallest inhibition zone was recorded on both *S. pneumoniae* ATCC49619 and *E. coli* ATCC25922, with a diameter of 9.5 mm. The positive control, chloramphenicol showed significantly larger zones on all test bacteria. The zone sizes were ranged from 17.5 to 35.5 mm. In contrast, the negative control methanol had no inhibitory effect on any of the test microorganisms.

Figure 1 : The inhibitory activity of sesame oil extract on *S. aureus* ATCC29213.

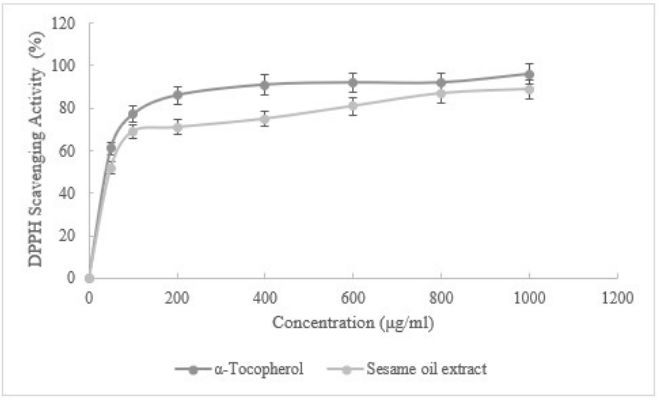

Figure 2 : The DPPH scavenging activity of sesame oil extract.

Broth microdilution assay

A wide range of MIC and MBC were recorded on all test bacteria (Table II). The MICs of the sesame oil extract ranged from 3.1 to 12.5 mg/mL, where the MBCs ranged from 6.3 to 25.0 mg/mL. The MBCs recorded were significantly higher than MIC. The lowest MIC of 3.1 mg/mL was recorded on *B. subtilis* ATCC6051, *S. aureus* ATCC29213 and *E. coli* ATCC8739, while the highest MIC of 12.5 mg/mL was recorded on *S. pneumoniae* ATCC49619 and MRSA ATCC43300. On the other hand, the lowest MBC of 6.3 mg/mL was recorded on *B. subtilis* ATCC6051 and *S. aureus* ATCC29213, while the highest MBC of 50.0 mg/mL was recorded MRSA ATCC43300.

Table II : MIC and MBC of sesame oil extract recorded on broth microdilution assay.

Test Bacteria	MIC (mg/mL)	MBC (mg/mL)
Streptococcus pneumoniae ATCC49619	12.5	25.0
Bacillus subtilis ATCC6051	3.1	6.3
MRSA ATCC43300	12.5	50.0
Staphylococcus aureus ATCC29213	3.1	6.3
Escherichia coli ATCC 8739	3.1	12.5
Escherichia coli ATCC 25922	6.3	12.5

Antioxidant activity

Figure 2 shows the DPPH scavenging activity of sesame oil extract. Eight different concentrations of sesame oil extract demonstrated different percentage of inhibition on DPPH free radicals. Basically, the DPPH scavenging activity was concentration dependent, where the extract's scavenging activity increased in a concentration-dependent way. The sesame oil extract at 1000 µg/mL showed the highest antioxidant activity. However, the scavenging activity of α-Tocopherol standard was higher compared to sesame oil extract. A significantly lower IC_{50} of 38.1 µg/mL was recorded for α-Tocopherol, compared to sesame oil extract that recorded the IC_{50} of 120.9 µg/mL.

DISCUSSION

Sesame oil is well known due to the presence of sterols, and antioxidative substances, such as sesamin, sesamolin, and tocopherols, which serve as nutraceuticals and give oil resistance to oxidative deterioration (3). In this study, sesame oil was extracted with methanol using liquid partitioning method. Extraction is a crucial step in the separation of bioactive compounds from their sources. Extraction methods and solvents have a significant impact on extraction yield and biological activity (13). Several parameters, including solvent, time, liquid-to-solvent

ratio, and temperature, are regarded as crucial in the extraction of bioactive substances (13). Liquid partitioning is a technique for separating bioactive compounds according to their relative solubilities in two immiscible liquids (14). Methanol is well-known for extracting antibacterial compounds from plants (13). Moreover, methanol is the most used extraction solvent due to its high polarity which could occur high extraction yield (15). Methanol was used in other studies (2,3). A significantly higher extraction yield of 31.6% was reported in another study on sesame seed oil using ultrasonic assisted extraction method (16). Therefore, ultrasonication using high frequency can be applied to improve the extraction yield of sesame oil.

Disc diffusion assay is an accurate standard method for determining antimicrobial susceptibility on selected test microorganism. This method is simple, repeatable, and dependable, with low supply and material costs (11). It is also more flexible than any other method, applicable to a wide variety of test microorganisms and substances, and requires no specific equipment (17). Therefore, this method was used because to determine the efficacy of sesame oil extract in inhibiting the growth of test bacteria. Bioactive compounds of plant origin are an alternative source of antibacterial agents. Based on the results, a wide range of diameters of inhibition zones were reported. This indicates different susceptibilities of the test bacteria to the extract. Disc diffusion assay is based on the diffusion of compounds impregnated in the paper disc. The diffusion produces a concentration gradient of bioactive compounds that creates a distinct zone of inhibition on pre-inoculated test microorganisms (17).

The antibacterial activity of the sesame oil extract was broad spectrum, as the extract inhibited both Gram positive and Gram negative bacteria. The observation was in agreement with previous studies. Sesame oil have been found to be effective against common skin pathogens such as *Staphylococcus* sp. and *Streptococcus* sp., as well as common skin fungi such as athlete's foot fungus (18). In another study using Saudi sesame oil, the extract also showed broad spectrum antibacterial activity, including *E. coli*, *S. aureus*, and S. pyogenes. The gas chromatography/ mass spectrometry analysis showed the antibacterial activity was due to the presence of sesamin and sesamol, the main fatty acids present in the extract (19). In addition, no clear zones were found in any of the negative controls (methanol), suggesting that the clear zones were caused by the bioactive compounds in the extract. The two major lignans found in sesame seeds oil were sesamin and sesamolin (3). Besides, sesamol at the concentration of 2 mg/mL was reported to inhibit the growth of *B. cereus*, *S. aureus* and *P. aeruginosa* (20). In contrast, the inhibitory activity was not reported on *P. aeruginosa* in this study at extract concentration of 1 mg/mL.

However, the sesame oil extract works better on Gram positive bacteria than Gram negative bacteria, where all the 4 Gram positive test bacteria were inhibited by the extract. Although Gram-positive bacterial cell walls are formed of thick layers of peptidoglycan, they do not block the passage of small to medium-sized molecules. Thus, it facilitates the penetration of low-molecular-weight compounds by providing an open network with a strong flux (21). Gram-negative bacteria, on the other hand, can circumvent the inhibitory effect of the compound through the expression of efflux pumps that remove the compound from the cell, the increased expression of antibiotic-inactivating enzymes, and permeability or target modifications (21,22).

Generally, the wide range of MIC and MBC indicate different susceptibilities of the test bacteria to sesame oil extract. The antibacterial activity was concentration-dependent. This is evidenced by the increased MBC observed for all test microorganisms, indicating that a higher concentration of sesame oil extract was required to kill the bacterial cells as opposed to suppressing their growth. Clinical and Laboratory Standard Institutes suggested that the clear broth becomes cloudy or turbid, and the presence of sedimentation indicates the growth of microorganisms for determination of MIC (23). In this instance, INT salt was used as the indicator of microbial growth (24). The transformation of yellow to purple indicates the presence of microbial growth; consequently, no colour change indicates the inhibitory effect of sesame oil extract.

In addition, the results of the broth microdilution assay were similar with those of the disc diffusion assay, which demonstrated that Gram-positive bacteria were more susceptible to sesame oil extract. The bacteria with the biggest inhibition zone in the disc diffusion assay also had the lowest MIC and MBC. The Gram-positive bacteria with the lowest MIC and MBC were *S. aureus* and *B. subtilis*. *S. aureus* is both a human commensal and pathogen. Around 30% of the global population is infected with the bacteria (25, 26).

In its oxidised state, DPPH is a stable free radical molecule with an absorbance of 515-520 nm. DPPH free radical scavenging assay is a relatively rapid and accurate method for assessing a substance's radical-scavenging activity (7). DPPH can receive an electron or radical hydrogen to generate a diamagnetic, stable molecule (27,28). The transition from violet to yellow indicates a decrease in

DPPH radical absorption. Calculating the sample concentration required to block 50% of radicals yielded the IC50 result (29). The lower the IC_{50} value of samples, the greater their antioxidant activity. In this work, the IC_{50} for sesame oil extract was shown to be 120.9 µg/mL. In a prior study, both roasted and unroasted sesame oil shown high antioxidant activity that increased with concentration. Also, the study found that roasting improved the antioxidant activity of sesame oil (30). The presence of sesamol, sesamol dimer, sesamin, sesamolin, sesaminol triglucoside, and sesaminol diglucoside in sesame oil contributed to its DPPH free radical scavenging activity (31). This study found a substantially lower IC_{50} value for sesame oil extract than previous studies (32,33). The antioxidant activity of sesame oil extract is regulated by a number of variables, including the origin of sesame seeds, the technique of sample treatment, the roasting temperature, the roasting time, and the storage conditions (18,34).

CONCLUSION

The present study showed that Ghee Hiang sesame oil extract showed significant antibacterial and antioxidant activities. The antibacterial activity was broad spectrum as the extract inhibited both Gram positive and Gram negative bacteria. DPPH scavenging activity of the extract was increased in a concentration dependent manner, and IC_{50} of 120.9 µg/mL was reported. Further investigations should be done to determine the bioactive entities present in the extract.

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