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 Turnitin Dosen 13
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trn:oid::1:3407524988

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bitorandAntimicrobialPotentialofMoringaoleiferaLseedsextract.pdf

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Bioactive Compounds, Tyrosinase Inhibitor and Antimicrobial Potential of *Moringa oleifera* L. seeds extract

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Abstract. *Moringa oleifera* L. seeds contain flavonoids, steroids and alkaloids, known to act as antioxidants. The purpose of this study was aimed at investigating the biologically active compounds, tyrosinase inhibition and antimicrobial activity of Moringa seeds against Gram-positive bacteria *Streptococcus pneumoniae* ATCC 49136 and Gram-negative bacteria *Escherichia coli* ATCC 25922. Identification of the chemical compounds was conducted using GC-MS technique, the tyrosinase inhibitory affects test was measured with spectrophotometry and the antimicrobial activity test was performed *in vitro* using the agar disc diffusion assay. The bioactive compound evaluation of Moringa seed extract confirmed the presence of 9 - Octadecenoic acid (50.67) ; Oleyl oleate (8.05%); 1-(1-Butoxy-2-propoxy)-2-propanol TMS derivative (4.12%) ; 4-Trifluoromethylbenzoic acid, tetradecyl ester (3.57%); 9-Octadecenoic acid (Z)-, oxiranyl methyl ester (3.26%). IC₅₀ values for inhibition of tyrosinase seed extract were 365.32 ppm and the kojic acid standard was 26.51 ppm. The antimicrobial activity was performed against bacteria as it showed inhibition zone. These results indicated that *Moringa oleifera* L. seed extract exerted potent antibacterial activity and is a promising candidate in the treatment of skin hyperpigmentation disorders. Keywords: Antibacterial, bioactive compound, *Moringa oleifera* L., tyrosinase inhibition

1 Introduction

Bioactive chemicals originating from plants have the ability to improve human health, and a sizable section of the world's population depends on medical nutrition therapy. Plant-based medicinal sources are widely utilized across various health programs and are expected to continue evolving for broader applications in the pharmaceutical industry. Moreover, the health-modifying properties of foods and their secondary metabolites play an essential role in reducing the risk of disease and promoting overall well-being [1].

One of the natural ingredients that have efficacy is Moringa seeds, contain phenolic compounds and flavonoids (flavonoids, steroids and alkaloids) that can serve as antityrosinase.

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Flavonoids, which encompass subclasses such as flavonols, isoflavones, flavan-3-ols, flavanones, chalcones, and others, exhibit a wide range of biological activities through diverse mechanisms. They are known for their strong antioxidant properties, tyrosinase inhibitory effects, and effective absorption of ultraviolet (UV) radiation [2].

Moringa oleifera L. is one of the vegetables that belongs to the family Moringaceae. This plant is a single genus family with 13 known species through research, a compound was found to contain many essential nutrients, for instance, vitamins. It has antibacterial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, and anti-inflammatory properties [3]. The greatest noticeable impact comes from antioxidants. Studies show that fresh moringa leaves have seven times more antioxidants than vitamin C. One group of antioxidants in moringa is called quercetin, which is part of the flavonoid family. The antioxidants in quercetin are four to five times stronger than those in vitamin C and vitamin E [4].

Hyperpigmentation is a pigmentary condition of the facial skin caused by an increase in melanogenesis. This process includes UV radiation, inflammation, hormones, and medication, which might cause darkening of the skin color. Sunray is an electromagnetic wave at the Earth's surface, consisting of several spectra: infrared light (>760 nm), visible light (400-760 nm), ultraviolet A (UVA) (315-400 nm), UVB (290-315 nm), and UVC (100-290 nm). It is highly hazardous, high-energy, and carcinogenic. Ozone depletion creates the potential for various diseases and health disorders [5].

Dark skin is a disorder in which the skin produces an excessive amount of melanin, which is the pigment that determines the skin. Increased melanin synthesis might result in localized pigmentation or black patches. Tyrosinase oxidation into dopaquinone is an important step in melanin formation. The route converts dopaquinone into dopa and dopachrome, which are subsequently converted into melanin. The synthesis process carried out by melanocytes involves a series of oxidation reactions, leading to the production of superoxide anions and hydrogen peroxide, which collectively contribute to maintaining melanocytes in a condition of oxidative stress [6].

Hyperpigmentation can be prevented through several approaches, including the application of sunscreen, the use of antioxidant agents, and the intake of essential vitamins and nutrients. The process of melanogenesis can be inhibited. Various studies have shown that many fruits and vegetables containing antioxidants can be natural skin brighteners. They act as tyrosinase inhibitors (inhibiting pigment production) [7].

Infectious diseases caused by Gram-positive and Gram-negative bacteria contribute to significant health problems worldwide. *Streptococcus pneumoniae*, commonly referred to as pneumococcus in medical microbiology, is one of the most prevalent Gram-positive pathogens responsible for pulmonary inflammation. Identified in the late 19th century as a leading cause of pneumonia, this bacterium is also associated with various other pneumococcal infections, particularly in children and the elderly, and is a major contributor to community-acquired respiratory illnesses beyond pneumonia [8].

Several factors contribute to the risk of developing invasive pneumococcal disease (IPD), including age—particularly in children under 2 years and adults over 65—as well as ethnicity, geographic region, the presence of underlying chronic conditions, and participation in group settings such as daycare centers *Streptococcus pneumoniae* is the leading cause of invasive diseases such as pneumonia, meningitis and sepsis [9].

Escherichia coli (*E. coli*) is a gram-negative rod-shaped bacterium responsible for various diarrheal diseases, such as traveler's diarrhea and dysentery. It is one of the most common pathogenic microorganisms associated with food-and waterborne infections. There are hundreds of known strains of *Escherichia coli* (*E. coli*), each capable of causing a wide range of diseases—from mild, self-limiting gastroenteritis to severe conditions such as renal failure and septic shock. *Escherichia coli* (*E. coli*) possesses the ability to evade the

host's immune defenses, contributing significantly to its pathogenic potential. Moreover, its increasing resistance to commonly used antibiotics further complicates treatment efforts. *E. coli* are Gram-negative bacteria that naturally inhabit the gastrointestinal tracts of humans and animals. In beef cattle, the majority of *E. coli* strains are non-pathogenic; however, certain strains can lead to diarrhea in calves. Similarly, while most *E. coli* strains found in humans are harmless, some pathogenic variants are capable of causing illness [10].

For this reason, we investigated the possible bioactive compound by GCMS to test the anti-tyrosinase was measured with spectrophotometry. These findings offer valuable insights into the antimicrobial against Gram positive bacteria *Streptococcus pneumoniae* ATCC 49136 and Gram-negative bacteria *Escherichia coli* ATCC 25922 were performed in vitro using the agar disc diffusion assay of 70% ethanol Moringa seeds extract. This research can increase the usefulness of *Moringa oleifera* L seeds across various domains and their applications within medicine.

2 Methodology

2.1 Tools and Materials

This study utilized various chemical substances, including moringa seed powder, 70% ethanol, distilled water, and Dragendorff, Mayer, and Wagner reagents. Additional solvents and reagents comprised methanol, ethyl acetate, 30% methanol, 30% ethanol, ammonia, chloroform, ether, and ferric chloride (FeCl_3). The materials employed in this study included the tyrosinase enzyme, L-3,4-dihydroxyphenylalanine (L-DOPA), hydrochloric acid (HCl) at a concentration of 2N, phosphate buffer solutions at pH 6.5 and 0.1 M at pH 7, a standard 6 M solution of 1,1,3,3-tetramethoxypropane (TMP), and sodium hydroxide. A variety of laboratory apparatus was utilized to support the experimental procedures, including measuring flasks, Erlenmeyer flasks, an Ohaus GA 200 analytical balance, a magnetic stirrer (exciter), a Memmert oven, test tubes, dropper pipettes, a laboratory knife, blender, porcelain crucibles, and an EYELA N-1100 rotary evaporator. Additional tools comprised a condenser bulb, Sanstat water bath, Mohr pipettes, filter paper, and a Genesys 10 UV-Visible spectrophotometer, with a wavelength detection range of 190–1100 nm. Complementary equipment included a graduated measuring cylinder, a Hettich Universal centrifuge (capable of operating at 0–6000 rpm), a plastic funnel, and a digital pH meter [11].

2.2 Drying Process of Test Material

Moringa oleifera L. seeds were sourced from the Kaliurang region in Yogyakarta, Indonesia, with a total of 3 kilograms collected. The seeds were thoroughly washed and then dried in an oven at 50°C for 4 to 5 days. After drying, the seeds were ground into a fine powder using a blender and subsequently sieved through a 60-mesh screen [11].

2.3 Moringa oleifera L. seeds Powder Extraction

A total of 5 grams of *Moringa oleifera* L. seed powder was weighed and extracted using a solvent at a ratio of 1:10 (w/v). The mixture was shaken continuously for 24 hours and then filtered using filter paper. This maceration process was repeated three times. The resulting macerates were subsequently concentrated using a rotary evaporator at 50°C to obtain the 70% ethanol extract [12].

2.4 Preparation of Samples for GC-MS Analysis

Moringa seeds 200 grams was soaked in 70% ethanol for 48 hours and extracted. The extract was re-extracted using chloroform to obtain chloroform soluble extract. This was centrifugated at 10.000 rpm for 20 minutes and the supernatant oil was subjected to GC-MS procedures [10]

2.5 GC-MS Procedures

Chemical substances were detected using the SHIMAZU Japan Gas Chromatography 5890-11, which was equipped with a fused GC column OV 101 covered with polymethyl silicon (0.25 mm x 50 m). The temperature is programmed from 80 to 200 degrees Celsius, with a rate of 5 degrees Celsius per minute and a duration of 20 minutes at 200 degrees Celsius. The FID temperature is 300°C, the injection temperature is 250°C, the carrier gas is nitrogen at a flow rate of 1 cm³/min, and the split ratio is 1:75. Mass spectrometric analysis was performed using a GCMS-QP 2010 Plus (Shimadzu, Japan), equipped with an injector maintained at 230 °C and operated under a carrier gas pressure of 100 kPa. The capillary column used measured 30 m in length with an internal diameter of 0.25 mm, and the flow rate was set at 50 m/min. Eluents were automatically introduced into the mass spectrometer, which operated at a detection voltage of 1.5 kV with a sampling interval of 0.2 seconds. Data acquisition was supported by a computer-integrated mass spectral database. Additionally, a HERMLE Z 233 M-Z centrifuge (Germany) was utilized. Analytical-grade reagents and solvents, including ethanol, were obtained from Merck (Germany) [10].

2.6 Tyrosinase Inhibitor Test

Dimethyl sulfoxide (DMSO) is a polar aprotic solvent with the chemical formula (CH₃)₂SO. It is known for ability to dissolve both polar and nonpolar compounds. DMSO was used to condense the extract at 10,000 ppm. The concentrated extract was dissolved in a 50 mM phosphate buffer with a pH of 6.5 to create the stock solution, which had a concentration of 600 mg/mL. Different concentrations of the extract—20, 200, 400, 800, 1600, and 3200 ppm. 30 µL of Tyrosinase enzyme (Sigma, 333 units/mL in phosphate buffer) was added to 200 ppm of kojic acid as a positive control, and the mixture was incubated for five minutes. After adding 110 µL of substrate (12 mM L DOPA), the mixture was incubated for 30 minutes at 37°C. A microplate reader was used to measure the solution's absorbance value at 492 nm in order to calculate the 50% inhibitory concentration (IC₅₀) and the percentage of inhibition. At a wavelength of 492 nm, the absorbance ratio of the sample without extract (A) with the addition of extract (B) was measured and computed to obtain:

$$\% \text{ Inhibition} = \frac{(\text{Abs Normal Control} - \text{Abs Blank}) - \text{Abs Sample}}{(\text{Abs Normal Control} - \text{Abs Blank})} \times 100 \%$$

The inhibition activity of the test sample was determined by the IC₅₀ values¹⁶ [13].

2.7 Determination of antibacterial activity

The agar well diffusion method was used to assess the extract of *Moringa oleifera* L.'s antibacterial qualities. To ascertain the extract's antibacterial effectiveness, the assay was tested against Gram-positive *Streptococcus pneumoniae* ATCC 49136 and Gram-negative *Escherichia coli* ATCC 25922. The solution of extract was prepared by dissolving 0.1 g of extract with 100 mL of solvent (distilled water and absolute ethanol) to produce 100 mg/mL. A series of extract solutions were prepared in distilled water at concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. For the minimum inhibitory

concentration (MIC) test, 25 μ L of each concentration was applied to sterile blank discs (6 mm in diameter), which were then placed onto Mueller-Hinton agar plates following the standard antimicrobial sensitivity testing procedure. The positive control used Ceftriaxone and the negative control used DMSO 20% for 24 hours incubation time at 37°C, and the inhibition zone of the extract was measured [14].

3 Results and Discussion

3.1 Phytochemical screening of Moringa seed extract

Phytochemical constituents present in the ethanolic extract of *Moringa oleifera* L. seeds were identified using Gas Chromatography–Mass Spectrometry (GC-MS) analysis. The identified compounds were characterized based on their retention time (RT), concentration (%), and compound names. A total of 13 compounds were detected, with details including their retention time, identification quality (Qlty), names, and respective concentrations (%) as presented in Table 1.

Table 1. Total ionic chromatogram (GCMS) of *Moringa oleifera* L. seeds

RT	Qlty	Name	Conc (%)
32.468	199	9-Octadecenoic acid, methyl ester,	1,28
34.116	93	9-Octadecenoic acid (Z)-, 2,3dih dro ro 1 ester	1,41
34.323	99	9-Octadecenoic acid 2,3dih dro ro 1 ester	50,67
35.274	27	1-Butoxy-2-propoxy)-2ro anol TMS derivative	4,12
35.384	74	9-Octadecenoic acid (Z)-, oxiran 1meth 1 ester	3,26
35.481	38	Fumaric acid, isopropyl tetrad 1 ester	1,30
35.853	55	4-Trifluoromethylbenzoic acid, tetrad 1 ester	3,57
36.633	45	(E)-Hexadec-9-enoic acid	1,86
37.205	47	4-Phenyl-3-penten-2-one toluenesulfon 1h drazone	1,61
37.501	59	Pyridine-3-carboxamide, oxime, N 2-trifluorometh 1 hen I -	1,81
39.611	53	Pyridine-3-carboxamide, oxime, N- 2-trifluorometh 1 hen I	1,78
43.769	91	Pyridine-3-carboxamide, oxime, N- 2-trifluorometh 1 hen I	1,56
48.720	42	Oleyl oleate	8,05.

The GC-MS result of the Moringa seeds extract in Table 1 showed the presence of thirteenn compounds. The bioactive compound evaluation of Moringa seed extract confirmed the presence of five compounds in high concentration are name 9 -Octadecenoic acid (50.67) ; Oleyl oleate (8.05%); 1-(1-Butoxy-2-propoxy) -2-propanol TMS derivative (4.12%); 4-Trifluoromethylbenzoic acid, tetradecyl ester (3.57%); 9-Octadecenoic acid (Z)-, oxiranyl methyl ester (3.26%). There are three of the same compounds in the different time presence Pyridine-3-carboxamide, oxime, N 2-trifluorometh 1 hen, with the different quality and concentration. Oleyl oleate compound in the last retention time (8,5%) is a versatile fatty acid ester with uses as an lubricant, conditioning agent in various industries or cosmetic [6].

3.2 Tyrosinase Inhibitor activity of Moringa seeds extract

The melanin synthesis within melanocytes depends on the tyrosinase enzyme that plays a vital role. By catalyzing the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), this enzyme's process produces dopaquinone, often referred to as DOPA chrome. This intermediate then undergoes polymerization to form melanin. The strategy for preventing

melanin accumulation in the skin, and treatment for skin pigmentation disorder depend on the inhibition of tyrosinase. The 70% ethanol extract in this investigation had the average value of measurement an IC_{50} value of 365.32 ppm, and the standard Kojic acid level was 26.51 ppm, indicating the potential to block the tyrosinase enzyme. Kojic Acid Naturally produced by fungus, kojic acid contains antioxidant and tyrosinase-inhibiting qualities (Figure 1)

The tyrosinase inhibitory activity of Moringa seed extract may result from its ability to compete with melanin substrates, such as L-DOPA, for binding at the enzyme's active site. Additionally, the extract may interfere with the chelation of copper ions at the enzyme's active site, thereby preventing the binding of copper to oxygen. This disruption can ultimately lead to the irreversible inactivation of the tyrosinase enzyme [15].

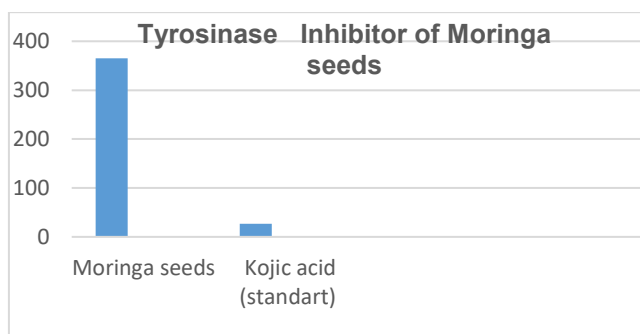


Fig. 1. The inhibition of the tyrosinase enzyme of Moringa seeds and Kojic acid (standart)

As shown in Figure 1, the tyrosinase inhibitory activity of the *Moringa oleifera* L. seeds extract. The inhibitory effect of this extract lower than Kojic acid (standart). The results demonstrated this seed extract possesses notable antityrosinase activity, highlighting its potential as a therapeutic agent for treating skin hyperpigmentation disorders.

3.3 The antimicrobial activity of Moringa seeds extract

The extract from Moringa seeds was made using 70% ethanol as an extracting solvent. Ceftriaxone was utilized as a positive control and DMSO as a negative control in antibacterial experiments. According to our findings, the extract from moringa seeds exhibited inhibitory effect against *E. coli* ATCC 25922 and *S. pneumoniae* ATCC 49136. Since it displayed zone inhibition, the action is less than that of the antibiotic Ceftriaxone (Figure 2 and 3). The measurement of the inhibition zone of Moringa seed extract against on Bacteria (Table 2 and 3).

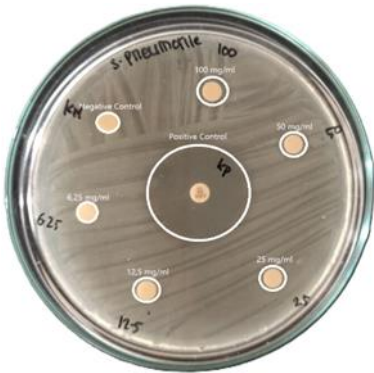


Fig. 2. The inhibition zone of Moringa seeds extract on Gram positive Bacteria *Streptococcus pneumoniae* ATCC 49136

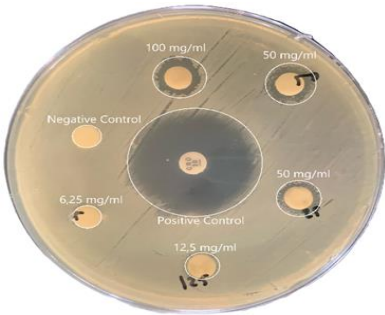


Fig. 3. The inhibition zone of Moringa seeds extract on Gram-negative Bacteria *Escherichia coli* ATCC 25922.

Agar well diffusion method was used. Finding from this research that *Moringa oleifera* L. seeds extract have antimicrobial activity on Gram-positive Bacteria *Streptococcus pneumoniae* ATCC 49136 and Gram-negative Bacteria *Escherichia coli* ATCC 25922.

Table 2. The Inhibition zone diameter of antimicrobial activity of Moringa seeds ethanol extract against *Streptococcus pneumoniae* ATCC 49136

Concentration of seed extrect (mg/mL)	Inhibition Zone (mm)				Average Zone of Inhibition (mm)
	1	2	3	4	
100	7	7.2	7.1	6.8	7.025
50	4.7	5	7.9	5	5.65
25	3.6	4	3.8	4.1	3.875
12.5	2.9	3	3	2.6	2.875
6.25	0	0	0	0	0
Positive Control (Ceftriaxone)	26	27	26	25.8	26.2
Negatif Control (DMSO 20%)	0	0	0	0	0

Table 3. The Inhibition zone diameter of antimicrobial activity of Moringa seeds ethanol extract against *Escherichia coli* ATCC 25922

Concentration of seed extract (mg/mL)	Inhibition Zone (mm)				Average Zone of Inhibition (mm)
	1	2	3	4	
100	7	7	6.2	7	6.8
50	5.2	5	4.9	5.3	5.1
25	4	4.5	4.1	4.3	4.225
12.5	3	3	3	2.8	2.95
6.25	2.3	2.5	2.8	2.3	2.475
Positive Control (Ceftriaxone)	25	25	24.6	25.1	24.925
Negative Control (DMSO 20%)	0	0	0	0	0

The present study revealed that Moringa seeds extract possess potential which are believed to be responsible for their antimicrobial activity. However, the action is not as high as that of the antibiotic Ceftriaxone. As both concentrations increased, the inhibitory activity tended to rise as well. Therefore, more research is needed to identify the molecules causing the antibacterial activity and the molecular mechanism behind the growth inhibitory action against *S. pneumoniae* and *E. coli*.

Recent research indicates that *Moringa oleifera* L. seed extracts exhibit antimicrobial properties, making them effective as natural antibiotics. These extracts have been shown to inhibit the growth of waterborne pathogens, foodborne illnesses caused by gram-negative bacteria *E.coli*, as well as infections linked to gram-positive bacteria such as *Streptococcus pneumoniae* that affect respiratory system.

4 Conclusions

The results demonstrated that the 70% ethanol extract of *Moringa oleifera* L. seed has possesses notable tyrosinase enzyme inhibitor activity with a total IC₅₀ values of 365.32 ppm and the Kojic acid standard was 26.51 ppm. Additionally, the extract has shown the antimicrobial activity was performed against both Gram-positive *Streptococcus pneumoniae* ATCC 49136 and Gram-negative *Escherichia coli* ATCC 25922 as it showed inhibition zone. Further studies are warranted to isolate and characterize the bioactive compounds responsible for these activities.

We would like to thank the Departement of Biochemistry and Microbiology, Faculty of Medicine, Universitas Kristen Indonesia for access to facilities.

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