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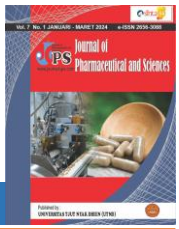

Antibacterial Activity of *Dendrophthoe pentandra* Mistletoe Leaf Extract on *Citrus microcarpa* Bunge Plants Against *Mycobacterium smegmatis*, *Escherichia coli* and *Salmonella typhi*

Aktivitas antibakteri ekstrak daun benalu *Dendrophthoe pentandra* pada tanaman *Citrus microcarpa* Bunge terhadap *Mycobacterium smegmatis*, *Escherichia coli* dan *Salmonella typhi*

Jabes Wolter Kanter [a]*, Jeane Mongi [a], Only Kalangi [a], Wilmar Maarisit [a], Douglas Natan Pareta [a], Christel Natanael Sambou [b], Selvana S. Tulandi [c]

[a] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Kristen Indonesia Tomohon, City Tomohon, Province North Sulawesi, Indonesia.*
[b] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sam Ratulangi Manado. Province North Sulawesi, Indonesia.*
[c] *Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Kristen Indonesia Tomohon, City Tomohon, Province North Sulawesi, Indonesia*

*Corresponding Authors: jabeskanter@gmail.com

Abstract

Diseases caused by bacteria infections are renowned for hurting human health and may become fatal when not treated with appropriate medical therapy. Meanwhile, several bacteria, including Mycobacterium smegmatis, Escherichia coli, and Salmonella typhi, are resistant to numerous antibiotics. Therefore, this study aimed to find new compounds from plants with antibacterial potential. The results showed that based on phytochemical screening, *Dendrophthoe pentandra* mistletoe leaf on *Citrus microcarpa* Bunge plants had compounds with antibacterial activity, namely alkaloids, flavonoids, tannins, and phenolics. According to Gas Chromatography-Mass Spectrometry (GC-MS) analysis, eight compounds have antibacterial properties, namely 2-Myristynoyl pantetheine; 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl; Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-; Ethyl iso-allocholate; a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate; tert-Hexadecanethiol; Sarreroside; and d-Mannose. *D. pentandra* mistletoe leaf extract had a better effect or activity on inhibiting the growth of *M. smegmatis* than *E. coli* and *S. typhi*. It was concluded that *D. pentandra* mistletoe leaf on *Citrus microcarpa* Bunge plants had antibacterial activity.

***Keywords*: Antibacterial activity, Dendrophthoe pentandra, Mycobacterium smegmatis, Escherichia coli, Salmonella typhi.**

Abstrak

Penyakit yang disebabkan oleh infeksi bakteri dapat berdampak buruk pada kesehatan manusia bahkan beresiko meninggal, jika tidak ditangani dengan terapi pengobatan yang tepat. Beberapa bakteri termasuk *Mycobakterium smegmatis, Escherichia coli, Salmonella typhi* mulai resisten dengan beberapa jenis obat antibiotik. Oleh karena itu, perlu dilakukan penelitian untuk menemukan senyawa baru obat dari tumbuhan yang memiliki potensi sebagai antibakteri. Hasil skrining fitokimia daun benalu *Dendrophthoe pentandra* pada tanaman *Citrus microcarpa* Bunge terdapat senyawa yang memiliki aktivitas sebagai antibakteri yakni alkaloid, flavonoid, tanin dan fenolik. Analisis *Gas Chromatography-Mass Spectrometry* (GC-MS) menunjukkan 8 senyawa sebagai antibakteri yakni 2-Myristynoyl pantetheine; 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl; Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-; Ethyl iso-allocholate; a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic

methylboronate; tert-Hexadecanethiol; Sarreroside; d-Mannose. Ekstrak daun benalu *D. pentandra* memiliki pengaruh atau aktivitas yang lebih baik pada penghambatan pertumbuhan bakteri *M. smegmatis* dari pada *E. coli* dan *S. typhi*. Berdasarkan hasil penelitian dapat disimpulkan bahwa daun benalu *D. pentandra* pada tanaman *Citrus microcarpa* Bunge memiliki aktivitas sebagai antibakteri.

Kata Kunci: Aktivitas antibakteri, Dendrophthoe pentandra, Mycobakterium smegmatis, Escherichia coli, Salmonella typhi.

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Introduction

Tuberculosis (TB) and infectious diseases caused by bacteria are still a threat to the community worldwide. [1][2] and in Indonesia. In general, TB is caused by *Mycobacterium tuberculosis* responsible for acute diseases in public health [3] and easily transmitted through the air due to droplet nuclei [4] from infected patients [5]. Infectious diseases caused by *Escherichia coli* and *Salmonella typhi* can also cause health problems for humans [6]. *E. coli* causes digestive diseases such as diarrhoea and vomiting [7], while *S. typhi* causes typhoid [8]. Based on the World Health Organisation (WHO) Global TB Report 2023, TB cases in Indonesia in 2022 are estimated to be 1,060,000 with an incidence of 385 per 100,000 population. This prevalence makes Indonesia one of the countries with the highest number of cases after India [9]. Diarrhea is among the infectious diseases of the digestive tract and according to WHO and UNICEF, there are around 2 billion cases worldwide. Approximately 1.9 million children under 5 die from diarrhea each year globally. Among all these deaths, 78% occur in developing countries, specifically in Africa and Southeast Asia. The 2018 Basic Health Research stated that the prevalence of diarrhea for all age groups ranges from 8% to 12.3% [10].

Mycobacterium tuberculosis and Mycobacterium smegmatis share approximately 75% of their genetic material, and a substantial number of virulence factors and biological mechanisms present in M. tuberculosis are also conserved in M. smegmatis [11]. Mycobacterium smegmatis is a rapidly growing, non-pathogenic species that has been widely adopted as a representative model in mycobacterial research, particularly for studies involving Mycobacterium tuberculosis [12]. Escherichia coli and Salmonella typhi are two Gram-negative bacteria commonly associated with intestinal and systemic infections in humans [13].

Antibiotics are the mainstay of modern medicine to control microbial infections [14] or stop bacteria growth [15]. Long-term use can cause resistance problems [16], hence, alternative antibiotics from plants are needed due to the properties of being safer, relatively cheap, and easy to obtain [17]. Knowledge of traditional medicinal plants is an inseparable and important part of indigenous cultures around the world [18]. WHO stated that 65% of the population in developed countries and 80% in developing countries have used traditional medicine [19]. The Minahasa community, from ancient times to the present, still uses plants such as leaves, roots, bark, stems, seeds, and fruit in medicine to cure diseases. The traditional medicine in this community [20] It is called Makatana, and the treatment process is known as *Mangundam*.

Manado lemon cui plant or *Citrus microcarpa* Bunge [21] is often used as a traditional medicine to treat cough [22]. The phytochemical content of the fruit juice contains compounds of alkaloid, flavonoid, terpenoid, and saponin groups [23], while the fruit and peel contain phytochemical compounds with antioxidant properties [24][25]. *Dendrophthoe pentandra* mistletoe [26] is a plant that lives as a parasite and takes essence and nutrition from the host. Several studies on the leaves of the parasitic plant Dendrophthoe pentandra hosted by different trees, such as the langsat tree, have shown that the leaf extract of Dendrophthoe pentandra exhibits antibacterial activity against Klebsiella pneumoniae [27], On mango trees, it exhibits antioxidant

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activity [28], Anti-cancer and antioxidant activity on langsat trees [29], anti-diabetic activity on Javanese wood trees [30], anti-inflammatory activity on cherry trees [31], and antioxidant activity on nutmeg trees [32], the presence of phytochemical substances is what makes these things happen. Utilization of natural resources from plants that have not been scientifically identified is the aim and target of research to discover new medicinal compounds, one example is the mistletoe leaves of *Dendrophthoe pentandra* on *Citrus microcarpa* Bunge, it is hoped that antibacterial activity tests will show better results than with patented or synthetic drugs. However, leaves of Citrus microcarpa Bunge plants have not been tested for chemical compound content and antibacterial activity. Therefore, this study aimed to test the antibacterial activity of *Dendrophthoe pentandra* mistletoe leaf extract on *Citrus microcarpa* Bunge plants against *Mycobacterium smegmatis, Escherichia coli* and *Salmonella typhi*.

Experimental Section

Mistletoe Leaf Samples

Dendrophthoe pentandra mistletoe leaf on *Citrus microcarpa* Bunge plants was taken from the community plant area in Tomohon City (1.324421 ° N 124.82254 ° E), North Sulawesi, Indonesia. Mistletoe plants are shown in Figure 1. Mistletoe leaf with a wet weight of 985 g and a dry weight of 657 g was then macerated for 3x24 hours using 96% ethanol and filtered to obtain the filtrate. The filtrate was evaporated to obtain a concentrated extract sample of 12.57 g.

Figure 1. (A) *D. pentandra* mistletoe on *Citrus microcarpa* Bunge plants. (B) Mistletoe leaves and flowers.

Phytochemical Screening and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Phytochemical Screening. About 2 grams of concentrated extract was analysed in the pharmaceutical chemistry laboratory of the Faculty of Mathematics and Natural Sciences, Indonesian Christian University of Tomohon, using several reagent compounds to determine the content of secondary metabolites.

Alkaloid Test. The extract solution was added with 10 mL of ammonia and 10 mL of chloroform. Furthermore, the solution was filtered into a test tube, and 10 drops of 2N H_2SO_4 were added to the filtrate. The mixture was shaken regularly and allowed to sit for a few minutes until two layers were formed. The upper layer was transferred into 3 test tubes, each containing 1 mL, and then added with a few drops of Mayer, Wagner, and Dragendorff reagents. Mayer's reagent produced a white precipitate, Wagner's reagent yielded a brown precipitate, and Dragendorff's reagent produced an orange precipitate. Flavonoid Test. The extract solution was added with 5 mL of ethanol and heated for 5 minutes in a test tube. Subsequently, a few drops of concentrated HCl and 0.2 g Mg powder were added, and the appearance of a dark red colour indicated a positive result. Saponin Test. The extract solution was added with distilled water until the entire sample was submerged, boiled for 2-3 minutes, cooled, and shaken vigorously. A positive result was indicated by the formation of stable bubbles/foam. Tannin Test. The extract solution was added with 2-3 drops of 1% FeCl3 solution. The formation of a bluish-black or green color indicated a positive result. Triterpenoid and Steroid Test. The extract solution was added with 2-3 drops of concentrated sulfuric acid. The presence of triterpenoids

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was indicated by the appearance of a red, orange, or purple color, while the formation of a blue color indicated steroids. *Phenolic Test*. The extract solution was placed into a test tube and then dripped with 5% FeCl_3 solution. The result was positive when there was a color change from yellow-brown to orange-brown.

Gas Chromatography-Mass Spectrometry (GC-MS). *D. pentandra* mistletoe leaf ethanol extract was analysed for chemical composition using GC-MS. About 4 g of concentrated extract was analysed by GC-MS at the Integrated Research and Testing Laboratory (LPPT) of Gajah Mada University, Yogyakarta. The sample was prepared by initially dissolving with a solvent, then EtOH 1 mL was added to a microtube, vortexed until homogeneous, and centrifuged at 9500 rpm for 3 minutes. The supernatant was taken in a GC vial, and the sample solution was ready to be injected. The separation used an HP-5MS UI column with a Length of 30 m, I.D. 0.25mm, Film 0.25µm, and Max. Temp. 325/350°C. The condition of Helium UHP (He) gas carrier was as follows Injector temperature 23°C, Operating mode split, Split flow 50 mL/min, Slit ratio 50, Front Inlet flow 1.00 mL/min, MS transfer line temp 250°C, Ion source temperature 200°C, Mass list range (amu) 40-500, and Purge flow 3 mL/min.

Bacteria Test

Concentrated extract from *D. pentandra* mistletoe leaf was made using a ratio of 1:1 (v/v) for extract and solvent. The disk diffusion or Kirby-Bauer method was used. [33] With 8 mm disc paper. Each disc of paper was spotted with 10 µl of the solution, including the mistletoe leaf extract, positive control, and negative control (distilled water). About 50 mg of extract [34]. It was diluted with 50 mL of 95% ethanol, then variations in the concentration of mistletoe leaf extract were made, including 12.5%, 25%, 50%, 75%, and 100% using the dilution formula M1.V1 = M2.V2. Distilled water of up to 10 mL was added for a 100% pure concentration of the extract solution. The disc paper was placed in a desiccator and left undisturbed for 24 hours to remove ethanol.

M. smegmatis bacteria 1 mL was grown in a liquid growth medium of 2 g Middlebrook 7H9 in 100 mL containing 2 mL glycerol and 1 mL Middlebrook OADC, along with a comparator of 0.5 MacFarland. Furthermore, the bacteria were cultured for 2 days/48 hours in an incubator at a room temperature of 37°C. About 2.5 g MiddleBrook 7H10 was weighed in 100 mL, and 2.5 mL glycerol was added to the container. The media to be used in the antibacterial activity test were sterilised in an autoclave for 15-30 minutes at a temperature of 121°C [35][36][37][38] with a pressure of 1 atm [39] Rifampicin 50 mg (450 g) was used as a positive control.

E. coli and *S. thypi* bacteria @1 mL were grown in a medium containing Nutrient Broth (NB) solution. About 4 g were dissolved in 100 mL of distilled water in an Erlenmeyer flask and then stirred. The bacteria were cultured and placed into a test tube, along with a 0.5 MacFarland comparator, and left for 24 hours in an incubator at room temperature, 37°C [40]. Nutrient Agar (NA) of 4 g was dissolved in 100 mL of distilled water in an Erlenmeyer flask and stirred. The media to be used in the antibacterial activity test were sterilised in an autoclave for 15-30 minutes at a temperature of 121°C. [41][42][43] with a pressure of 1 atm [39]. About 50 mg of ciprofloxacin (500 g) was used as a positive control.

The measurement of the inhibition zone diameter was carried out using the formula:

$$\mathbf{d = (A + B + C) / 3}$$

Where **d** represents the average inhibition zone diameter, **A** is the vertical diameter, **B** is the horizontal diameter, and **C** is the diagonal diameter. This calculation was performed to obtain a more representative average value of the inhibition zone formed.

Statistical Analysis

The test was repeated 3 times, and the data were analysed using a one-way analysis of variance (ANOVA) [44][45]. Data were presented as mean and standard deviation (SD), with a statistically significant level set at P value <0.05[33]. The analysis was carried out using the IBM SPSS Statistics version 22 application.

Results and Discussion

Secondary metabolites in plants are an important source of active drug compounds. [46] with great potential [47] to treat various types of diseases [48], including antimicrobials [49][50]. The phytochemical

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screening results showed that *D. pentandra* mistletoe leaf extract in *Citrus microcarpa* Bunge plants contains secondary metabolites, namely alkaloids, flavonoids, tannins, saponins, and phenolics, using several test solvents as in Table 1 below.

Table 1. Phytochemical screening results

Compound Group	Result	Color Change
Alkaloids	+	Orange (Dragendorf)
	+	Brown (Wagner)
	+	White (Meyer)
Flavonoids	+	Dark Red
Tannins	+	Green/Bluish
Saponins	+	Bubbles/Foam
Steroids	-	No colour change
Triterpenoids	-	No colour change
Phenolics	+	Orange-brown

Compounds with antibacterial potential are alkaloids, flavonoids, tannins, and phenolics. [40][44][33][51][52][53]. These compounds have various benefits; for example, alkaloids possess pharmacological activity as antibacterials, anticancers, and antivirals. Flavonoids act as antibacterials by inhibiting bacteria cell metabolism and can help lower total cholesterol levels while also preventing hypercholesterolemia. Tannins perform antibacterial functions by deactivating enzymes in bacteria, while saponins have pharmacological activity as immunomodulators, antioxidants, anti-inflammatories, antivirals, and anticarcinogens. Phenolics have pharmacological activity such as antibacterial, anti-inflammatory, antiviral, antioxidant, and Anti-cancer. [54]. GC-MS results of *D. pentandra* mistletoe leaf extract on *Citrus microcarpa* Bunge plants show data as in Figure 2:

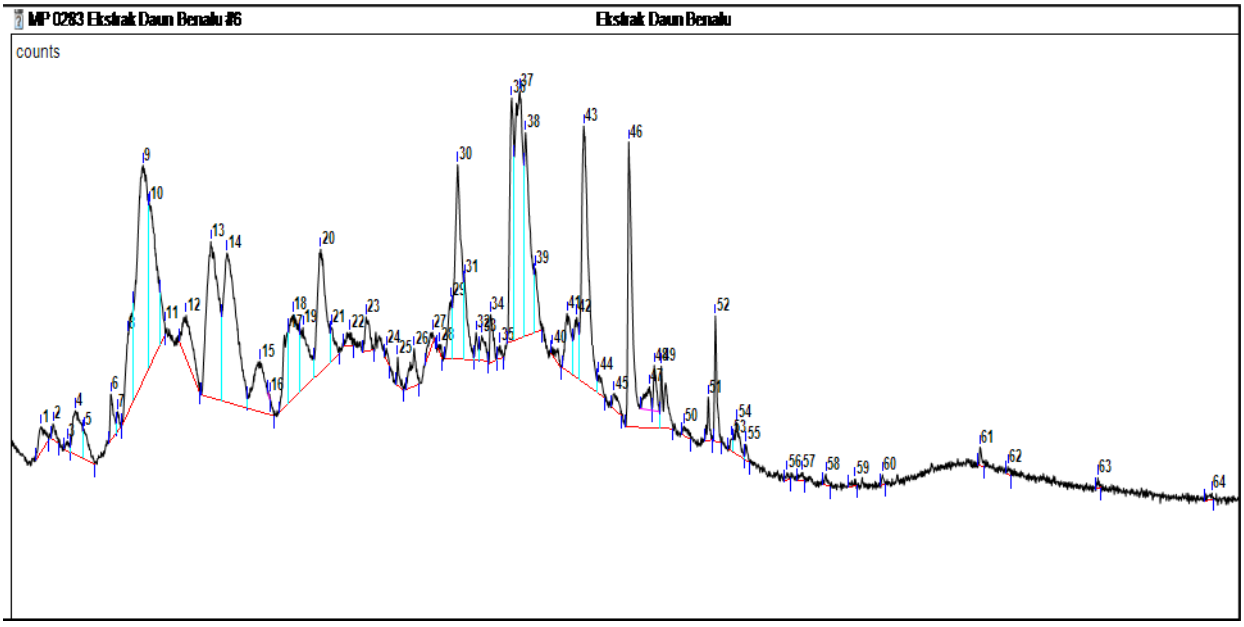

Figure 2. GC-MS Chromatogram of bioactive compounds in *D. pentandra* mistletoe leaf extract

The analysis results of biologically active compounds using GC-MS show the presence of 65 possible compound components in hits 1, 2, and 3. The five highest compound components include Desulphosinigrin [no-*37*]; 2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl [no-*36*];

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Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl- [no-*43*]; d-Gala-l-ido-octonic amide [no-38]; Lidocaine [no-*46*], as in the table 2 below:

Table 2. Highest compound components

No.	Compound Name	Molecular Formula	Molecular Weight	Resting Time	Rel. Area %
1	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	15.03	7.22
2	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$	206	14.46	3.76
3	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	$C_{14}H_{24}O_3$	240	16.42	6.87
4	d-Gala-l-ido-octonic amide	$C_8H_{17}NO_8$	255	15.16	4.53
5	Lidocaine	$C_{14}H_{22}N_2O$	234	17.40	6.11

There are eight compounds with antibacterial potential in hits 1, 2, and 3, including 2-Myristynoyl pantetheine. [55], 2H-Indeno[1,2-b]furan-2-one,3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl [56][57][58], Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-[59], Ethyl iso-allocholate [60][61], a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate[62][63], tert-Hexadecanethiol [64][65][66][67], Sarreroside [68], d-Mannose [69][70]. Additionally, there are two groups of Hexadecanoic acid in hits 1 and 2 with antibacterial potential, namely Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8amethanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester,[1aR-(1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-[71][72], and Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester [52][55]. The GC-MS analysis results indicate compounds with potential antibacterial activity, which will likely significantly impact the antibacterial testing against *Mycobacterium smegmatis*, *Escherichia coli*, and *Salmonella typhi*. These compounds are presented in Table 3 below.

Table 3. Compounds with antibacterial potential

No.	Compound Name	Molecular Formula	Molecular Weight	Resting Time	Rel. Area %	Hit. 1/2/3
1	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	13.29	0.08	1
2	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$	206	14.46	3.76	1
3	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	$C_{11}H_{18}N_2O_2$	210	16.74	0.30	1
4	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	25.03	0.16	1
5	a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate	$C_{13}H_{26}BNO_6Si$	331	25.63	0.08	1
6	Hexadecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	$C_{36}H_{58}O_6$	586	31.56	0.12	1
7	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	19.28	1.12	2
8	Sarreroside	$C_{30}H_{42}O_{10}$	562	21.68	0.12	2

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9	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	22.91	0.08	2
10	d-Mannose	$C_6H_{12}O_6$	180	17.95	0.54	3

Inhibition of bacteria growth caused by *D. pentandra* mistletoe leaf extract [73] In *Citrus microcarpa* Bunge, the plants were measured based on the clear inhibition zone around the disc paper. Based on the category of Davis and Stout (1971) [73]The inhibition zone diameter of *M. smegmatis* for concentrations of 12.5% and 25% was categorised as strong, while 50%, 75%, and 100% were in the very strong category. For *E. coli* and *S. typhi*, the inhibition zone diameter from the five test concentrations was all categorised as strong, ranging between 10-20 mm. The results of the inhibition zone observations and calculations of antibacterial activity are shown in Figure 3 below.

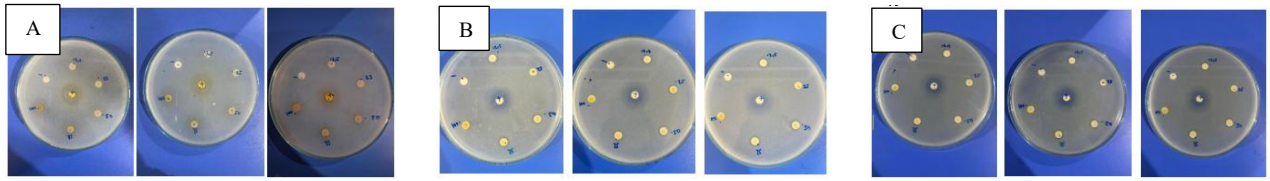

Figure 3. (A). *Mycobacterium smegmatis.* (B). *Escherichia coli.* (C). *Salmonella typhi*

Analysis of Variance (ANOVA) test results showed that the P value = 0.00 <0.05 from the three test bacteria, indicating a significant Influence between the independent and dependent variables, as in Table 4. Qualitatively, the extract test results correlated with the concentration [74] Experimental results confirmed that the lowest concentration tested, 12.5%, was sufficient to elicit antibacterial activity.

Table 4. Statistical analysis results

No.	Treatment Concentration	Bacteria			*P< 0.05*
		M. smegmatis	*E. coli*	*S. typhi*	
		Mean ± SD			
1	12.5%	13.85 ± 1.34	12.57 ± 0.88	10.90 ± 2.15	
2	25%	17.09 ± 1.02	10.16 ± 1.10	10.75 ± 1.95	
3	50%	21.27 ± 0.97	11.87 ± 0.33	11.19 ± 0.50	
4	75%	23.50 ± 0.55	11.97 ± 0.33	13.86 ± 1.82	***0.000***
5	100%	24.99 ± 1.00	16.94 ± 0.59	13.86 ± 1.47	
6	Positive Control	28.85 ± 0.79	30.55 ± 1.21	24.88 ± 0.33	
7	Negative Control	0	0	0	

The results of statistical analysis using one-way ANOVA indicate that the clear zones or inhibition zones formed, calculated using the diameter of the inhibition zones vertically, horizontally, and diagonally divided by three, show variation in diameter according to the concentration of the extract used in the tests. For the bacterium *M. smegmatis*, at a concentration of 12.5%, the mean value was 13.85 with a standard deviation (SD) of 1.34; for the bacterium *E. coli*, at a concentration of 12.5%, the mean value was 12.57 with an SD of 10.88; and for the bacterium *S. typhi*, at a concentration of 12.5%, the mean value was 10.90 with an SD of 2.15. For the bacterium *M. smegmatis* at a concentration of 100%, the mean value was 24.99 with an SD of 1.00; for the bacterium *E. coli* at a concentration of 100%, the mean value was 16.94 with an SD of 0.59; and for the bacterium *S. typhi* at a concentration of 100%, the mean value was 13.86 with an SD of 1.47. The results of the ANOVA test statistical analysis show a p-value < 0.05 or 0.00 < 0.05 for all three bacteria, indicating that there is a significant relationship between the concentration of the Dendrophthoe pentandra extract samples and the clear/inhibition zones formed, implying that the compounds in Dendrophthoe pentandra have a substantial effect on the growth of the three bacteria.

According to the categorization by David and Stout (1973), the diameter of the inhibition zone at concentrations of 12.5% and 25% is categorized as strong (diameter 10-20mm), while at concentrations of 50%, 75%, and 100% for the treatment of *M. Smegmatis* bacteria, it is categorized as very strong (diameter > 20mm). Meanwhile, the diameter of the inhibition zone at concentrations of 12.5%, 25%, 50%, 75%, and 100% (diameter 10-20mm) for the treatment of *E. coli* and *S. typhi* bacteria is all categorised as strong (diameter 10-20mm). This

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indicates that the bioactive compounds present in the mistletoe leaf extract of *Dendropthoe pentandra* affect the growth of bacteria. The average inhibition value of each concentration in *D. pentandra* mistletoe leaf extract showed that the higher the concentration of the extract given, the greater the inhibition of *M. smegmatis*, *E. coli*, and *S. typhi* growth. However, in *E. coli* and *S. typhi*, the 25% concentration formed a smaller inhibition zone than the 12.5%. This may be attributed to biological variability factors occurring during the testing process. The increase in the inhibition zone is illustrated in Figure 4 below.

M. smegmatis is a nonpathogenic species that quickly grows but is environmentally friendly when cell culture is carried out in the laboratory as a model of *M. tuberculosis* [75]. Mycobacterium have a different cell envelope structure than Gram-positive bacteria, which are proven to contain inner and outer membranes. [76]. Rifampicin is an antibiotic drug. [77] used as the first line in TB treatment [78][79][80], which works by inhibiting RNA polymerase. The inhibition of bacterial growth caused by the extract of D. pentandra leaves at a concentration of 100%, with a mean value of 24.99mm, is almost comparable to rifampicin as the positive control, which has a value of 28.85mm (Table 4). This may be due to the presence of one or more phytochemical compounds [81] Active against *M. smegmatis*.

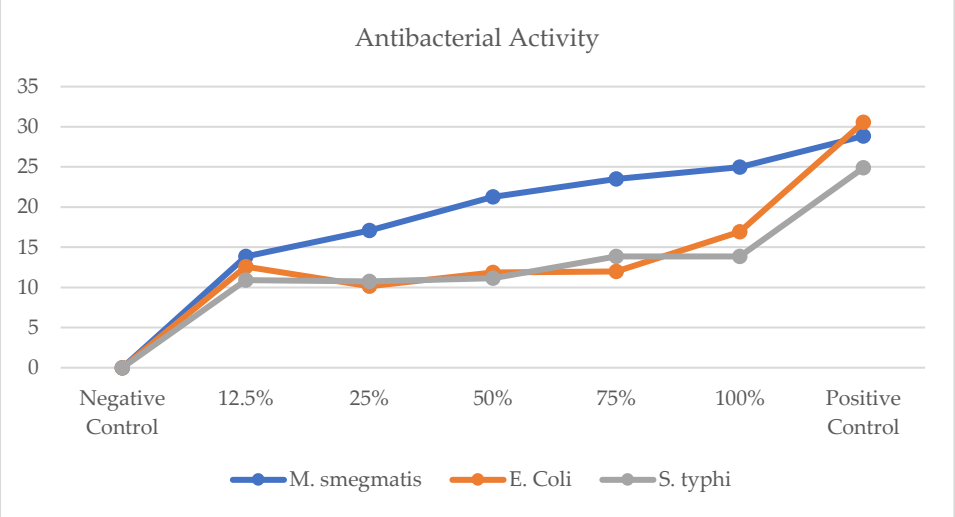

Figure 4. Graph of the increase in the inhibition zone

E. coli and *S. typhi* are pathogenic gram-negative bacteria [82] That can cause disease and death in humans. [34][83][84]. Meanwhile, ciprofloxacin is an antibiotic drug used in the treatment of bacteria infections. [85][86]. These bacteria may become resistant. [74][87] to antibiotics such as ciprofloxacin [88] and have replicated in human cells [89]. Phytochemical compounds or secondary metabolite content in *D. pentandra* mistletoe leaf extract, such as alkaloids, flavonoids, tannins, and phenolics, are factors that inhibit bacteria growth. [90] Or play a role in synthesis [91] or inhibit DNA ligation.

Possible antibacterial mechanisms of secondary metabolite compounds are as follows. Alkaloids interfere with cell wall mechanisms and inhibit deoxyribonucleic acid (DNA) synthesis, while flavonoids change microbial cell membranes as well as impede energy metabolism and nucleic acid synthesis. Tannins work to deactivate microbial adhesins, enzymes, and bacteria cell envelope transport proteins, and phenolics disrupt microbial cell membranes. This study is the first to report that *D. pentandra* mistletoe leaf extract in *Citrus microcarpa* Bunge plants inhibits the growth of *M. smegmatis, E. coli,* and *S. typhi*. GC-MS analysis results also show the presence of several compounds with potential antioxidant, Anti-cancer, anti-inflammatory, and other therapeutic activities, which require further study. Additionally, fractionation using solvents such as n-hexane, ethyl acetate, and methanol should be carried out to isolate specific compounds based on their polarity characteristics.

The three bacteria, *M. smegmatis*, *E. coli*, and *S. typhi*, have a significant adverse impact on human health in environmental microbiology. *M. smegmatis* requires intensive management to prevent fatal impacts on human respiratory and lung tissues. Similarly, *E. coli* and *S. typhi*, commonly found on human skin tissue and in early childhood snacks or the community environment, also need careful attention. Since the leaves of Dendropthoe pentandra have shown significant effects, further research is required to identify which compounds are most potent as antibacterial agents.

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Conclusions

In conclusion, *D. pentandra* mistletoe leaf extract on *Citrus microcarpa* Bunge plants has antibacterial activity against *Mycobacterium smegmatis*, *Escherichia coli*, and *Salmonella typhi* due to the secondary metabolites or bioactive compounds such as alkaloids, flavonoids, tannins, and phenolics. Concentrated mistletoe leaf extract provided stronger activity or Influence on *M. smegmatis* than *E. coli* and *S. typhi*, as demonstrated by the formed inhibition zone diameter, comparable to that of the patented or synthetic drugs used as positive controls. This indicates that the bioactive compounds possess specificity, resulting in differential activity across bacterial species.

Conflict of Interest

The authors declare that they have no conflicts of interest related to the research, authorship, or publication of this article.

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Supplementary Materials

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