

Evaluation of *In-vitro* Antibacterial activity of extracts of *Horsfieldiaryghedhi* grown in Sri Lanka.

ABSTRACT

Aims:The creation of new naturally occurring medication mostly depends on a multidisciplinary effort to discover new compounds. Antibiotic resistance has been acquired by some bacteria strains. As a result, new anti-bacterial drugs are urgently acquired to combat bacterium resistance. This study aims to evaluate the *in vitro* antibacterial activity of methanol and aqueous leaf, bark, and combination extracts of the plant *Horsfieldiaryghedhi*.

Methodology:Aqueous and methanol extracts were obtained from the cold maceration method. *In vitro*, antibacterial activity of methanol and aqueous leaf, bark, and combination extracts were determined against gram-negative bacteria *Escherichia coli* (ATCC[®] 25922) and gram-positive bacteria *Staphylococcus aureus* (ATCC[®] 25923). The anti-bacterial assay for different concentrations of each extract was conducted through the well-plate method and Gentamycin was used as the positive control. And the zones of inhibition were measured.

Results:Methanol leaf and combination extracts of *Horsfieldiaryghedhi* have shown a positive antibacterial response at their highest concentrations against gram-positive bacteria *Staphylococcus aureus* (ATCC[®] 25923). Thus, this study's results indicate that *H. iryaghedhi* plant extracts show a negative response against gram-negative bacteria, *E. coli*(ATCC[®] 25922)at the experimented concentrations.

Conclusion:The study concludes that extracts of *H.iryaghedhi* extracts should be further analyzed for their antibacterial activity using different solvent extracts with different concentrations.

Keywords: *Horsfieldiaryghedhi*, Anti-bacterial, *Escherichia coli* (ATCC[®] 25922), *Staphylococcus aureus* (ATCC[®] 25923), well plate method

1. INTRODUCTION

Antibacterial agents and specific antibiotics have played a crucial role in saving humanity from the debilitating effects of numerous infectious diseases and contagious agents. Despite their continued usefulness, the natural resistance, overuse, and considerable misuse of these agents have led to the development of resistance among many infectious organisms, creating an urgent global need for alternative solutions. As medicine continues to advance, with the advent of new medications and technological breakthroughs in surgery, bacteria continue to evolve and adapt, necessitating the discovery of new compounds, their approval by health regulators, and their manufacture on a large scale. Therefore, it is imperative to explore new avenues to combat antimicrobial resistance and to develop alternative solutions that can meet the evolving needs of the healthcare industry(1).

The current state of antibiotic discovery is not keeping pace with the escalating levels of antibiotic resistance, a pressing global health concern. Factors such as an unfavorable regulatory climate, scientific challenges, low financial returns, and industry consolidation, have prompted many pharmaceutical companies to withdraw from antibiotic drug development. To address the current crisis and deliver drugs for challenging disease conditions, it is crucial to increase the productivity of the antibiotic drug discovery pipeline. One of the primary scientific obstacles that must be overcome is the efficient delivery of molecules into gram-negative bacterial cells while evading efflux. Screening programs have been initiated to address these challenges. Despite the challenges facing antibiotic discovery, natural product research still holds promise as a source of new molecules for drug discovery(2).

Plants have long been recognized as a valuable source of compounds for the treatment of infectious diseases. Antibiotics, whether natural or synthetic/semisynthetic organic molecules, have demonstrated efficacy against a wide range of microorganisms such as bacteria, fungi, and protozoa. Plants produce complex suites of compounds known as secondary metabolites that provide them with the ability to defend against pathogens. These compounds also have the potential to offer protection to humans against infectious agents. As an alternative or complementary approach to antibiotics, naturally occurring plant-based compounds are increasingly being explored. In contrast to antibiotics, plant-based compounds demonstrate potential antimicrobial activities that can be used either alone or in combination with antibiotics to address the current crisis of antibiotic resistance(3).

Throughout history, medicinal plants have been employed as a source of healing in communities worldwide. To this day, they remain a significant source of drug discovery, as they have contributed to the development of synthetic pharmaceuticals. Medicinal plants are considered to be abundant sources of constituents that can be utilized in the development of pharmacopeial, non-pharmacopeial, or synthetic drugs. According to the World Health Organization (WHO), over 80000 species of higher plants have been documented for their therapeutic use out of the world's 25 million species. Furthermore, it is estimated that roughly 21000 plant species have the potential to be utilized as therapeutic plants(4).

Herbal medicines have been acknowledged for their effectiveness, minimal side effects, and affordability in comparison to Western medicines. Medicinal plants are a valuable natural resource that has been utilized in the development of novel drugs. With the increasing global recognition of the positive effects of traditional medicine, folk medicines may emerge as a new phase of the medical system for treating human ailments in the coming centuries(5).

The Myristicaceae family is a group of flowering plants that is indigenous to Africa, Asia, the Pacific islands, and the Americas. It is commonly referred to as the nutmeg family, named after its most well-known member, the *Myristica fragrans*, which is the source of nutmeg and most of the species in this family. *Horsfieldia*, also known as the Malaboda tree, is a fast-growing flowering plant that is native to Sri Lanka and belongs to the Myristicaceae family. These trees typically grow to a height of between 10 and 20 meters and are prevalent in Sri Lanka's wet zone, particularly along the borders of paddy fields, margins of water streams, and rivers. The Myristicaceae family comprises 520 species of trees and shrubs that are found in tropical forests around the world. These plants are fragrant, perennial, and have a distinct aroma that is widely utilized for medicinal purposes. They are known to treat stomach ulcers, indigestion, liver disease, and act as an emmenagogue, nerve tonic, diuretic, diaphoretic, and aphrodisiac. Moreover, essential oils extracted from Myristicaceae contain anti-fungal and anti-bacterial

properties in some genera, such as *Virola*, while the dark red resin of the tree bark includes multiple hallucinogenic alkaloids(6).

Horsfieldia iryagedhi, which is commonly known as RUK, is a plant used in treating infections and other various medical conditions and this plant may have many more other medicinal uses which are yet to be found.



Figure 1: *Horsfieldia iryagedhi* flowers Figure 2: *Horsfieldia iryagedhi* leaves and stem

2. MATERIALS AND METHODOLOGY

2.1 Materials

2.1.1 Chemicals

Absolute Methanol (99% v/v) (Sigma Aldrich), Hexane (99% v/v) (Sigma Aldrich), Diclofenac Sodium (DC) (98% w/w) (standard) powder was collected from the State Pharmaceutical Manufacturing Corporation of Sri Lanka. Gentamycin (80mg/2ml) (SPC), Phosphate buffered saline (pH 6.4), Normal saline (0.9% w/v) (B.BRAUN), Barium Chloride (1.175% w/v BaCl₂.2H₂O), Sulphuric Acid (1% v/v), Muller Hinton Agar (HiMedia Laboratories Pvt.Ltd).

2.1.2 Instruments and Equipment

Rotary evaporator (HAHNSHIN Scientific-model no-H2005V,SR no;V-00449), Analytical balance (ACET, Model No: CY-224C, S/N: R600008446), Laboratory vortex (Huma Twist, REF: 17175, S/N:VB192AH011635), Autoclave machine (TOMYKOGYO co. Ltd, Model-SX-500, SR no; 49133064), Hot air oven (BOV-V2225F with RS485), Incubator (CLW 240 IG SMART), Domestic grinder(INNOVEX, Model: IMG O10).

The study was carried out in the Pharmacy Skill Laboratory, Faculty of Allied Health Sciences in General Sir John Kotelawala Defence University, Werahera.

2.2 Methodology

2.2.1 Sample collection

About 1500 g of each matured, fully expanded bark and leaves of *Horsfieldiaryaghedhi*(RUK) were collected in fresh conditions during daytime from Mattegoda Osu uyana, Colombo District in Western Province of Sri Lanka (Coordinates: 6°48'05.4"N, 79°58'37.6"E).

2.2.2 Identification and Authentication of the plant

The properly dried and pressed specimen was sent to the Pharmaceutical Botany division at Ayurveda Research Institute, Nawinna, Sri Lanka for authentication. The identification and authentication processes were performed by voucher specimens deposited at the herbarium.

2.2.3 Preparation of crude plant material extracts

Selected plant materials of *Horsfieldiaryaghedhi*were thoroughly cleaned using running tap water and dried until a constant weight was obtained. The dried plant materials were then powdered using a domestic grinder to obtain a coarse powder. Well-dried and blended coarse powder samples of plant materials were taken for the extraction procedure.

2.2.3.1 Methanol extraction procedure

The powdered plant materials (20g) of each leaf, bark, and combination were suspended in 160 ml of 99.9% methanol in separate closed glass bottles. These were kept for 7 days with occasional shaking in an orbital shaker. The extracts were filtered through a double-layered muslin cloth and Whatman No 1 filter paper using the Rotary evaporator (HAHNSHIN Scientific-model no. H-2005V, SR no: V-00449) at 40°C. Methanol was evaporated from the filtrate to get the dry residue. For further evaporation, the sample was kept at room temperature for 2 hours(7).

2.2.3.2 Aqueous extraction procedure

The powdered plant materials of each 20g of leaves, bark, and combination were suspended in 160 ml of distilled water in separate closed glass bottles. These were kept for 7days with occasional shaking in an orbital shaker. The extracts were filtered through a double-layered muslin cloth and Whatman No 1 filter paper. A rotary evaporator was used at 60°C to get the residue(8).

2.2.4 Evaluation of in vitro antibacterial activity

2.2.4.1 Collection and sub-culturing of test microorganisms

Pathogenic strains of *Escherichia coli* (ATCC® 25922) and *Staphylococcus aureus* (ATCC®25923) were obtained from the Medical Research Institute, Colombo 08, and Sri Lanka. They were subcultured on Mueller-Hinton agar plates and maintained at 2-4°C to be used for further studies.

2.2.4.2 Preparation of test solutions

Stock solutions were prepared by dissolving 0.25g of each plant extract (methanol leaf, methanol bark, methanol combination and aqueous leaf, aqueous bark, aqueous combination) in 50 mL of distilled water separately. Then from each, 4 mL of solution was dissolved in distilled water to get 20 mL of solution. The concentration of the stock solution was considered as 1000µg/mL. Concentration series for the test solutions were prepared as 1000µg/mL, 500µg/mL, 250µg/mL and 125µg/mL(9).

2.2.4.3 Preparation of Gentamycin antibiotic for positive control

In this study, Gentamycin which was commercially available as a 40 mg/mL IV injection vial was used as the positive control.

The standard was prepared by dissolving 0.25mL of Gentamycin, in 50ml of distilled water. Then 4 ml from the above solution was dissolved in distilled water to get 20mL of solution. The initial concentration of the standard was obtained as 1000µg/mL. The concentration series for the standard solution was prepared as 1000µg/mL, 500µg/mL, 250µg/mL, and 125µg/mL(10)

2.2.4.4 Preparation McFarland Standards

A 0.5 McFarland standard is equivalent to a bacteria suspension containing between 1×10^8 and 2×10^8 CFU/mL of relevant bacteria. A 0.5 McFarland standard was prepared in the laboratory, 0.5mL aliquot of BaCl₂ was added to 99.5 mL of H₂SO₄ with constant stirring to maintain a suspension (11).

2.2.4.5 Preparation of bacterial broth

Two to three bacterial colonies were obtained from subcultures using a sterile inoculating loop and dissolved in 25 mL of 0.9% normal saline under aseptic conditions. Then the bacterial suspensions were compared using a Wickerham card with the previously prepared McFarland Turbidity Standard. The turbidity of bacterial suspension was adjusted to get equivalent with 0.5 McFarland Standard. Turbidity of 0.5 McFarland Standard is equivalent to that of a bacterial suspension of 1.5×10^8 CFU/mL (12).

2.2.4.6 Preparation of culture media

To prepare the culture media, 19g of commercially available Mueller-Hinton agar (brand) was measured using an electric balance and transferred to a 500 mL conical flask. 500 mL of distilled water was added gradually to dissolve agar while stirring. Flasks were prepared as above and were autoclaved using an autoclave machine (TOMYKOGYO co. ltd, Model-SX-500, SR no; 49133064) for 15 minutes at 121° C temperature under 15 lbs pressure. All the glass wear needed was kept in a hot air oven (BOV-V2225F with RS485) at 121°C temp for 2 hours.

2.2.4.7 Screening for antimicrobial activity using agar well diffusion method

11.11 mL of each bacteria suspension was added into 500 ml Mueller-Hinton agar-containing flasks. The above was done under sterile aseptic conditions. Initially, the thin layer of Mueller-Hinton agar was added and allowed to solidify. Six sterilized Aluminium cylinders with dimensions of 8.0mm×6.0mm×6.0mm and an inner diameter of 6.0mm, open on both ends were kept on a previously solidified agar layer. Cylinders were placed according to a template placed under the Petri plate which had uniform gaps between cylinders. Bacteria containing culture media was mixed and poured into the 130mm sterilized Petri plate under aseptic conditions and allowed to cool down to settle the agar. Forceps were sterilized by cleaning them with sterile alcohol and it was used to remove the Aluminium cylinders from completely solidified agar. All procedures were carried out under strict aseptic conditions.

Each prepared plate with seven wells was filled with 200µl of four concentrations (1000µg/mL, 500µg/mL, 250µg/mL, and 125µg/mL) of each plant extract, positive control, and negative control. The same process was repeated to the Gentamycin concentration series (1000µg/mL, 500µg/mL, 250µg/mL, and 125µg/mL) as well.

All the plates were incubated (CLW 240 IG SMART) at 37°C for 24 hours. After incubation, the diameter of the inhibition zone was measured using a Vernier caliper, and measurements obtained from three trials were calculated to obtain the mean zone of inhibition.

2.2.4.8 Determination of IC50 values

IC50 values for each extract and standard were determined by the method of non-linear regression fit, sigmoidal, 4PL, X is log (concentration) using Graph pad prism 9 (version 9.3.1).

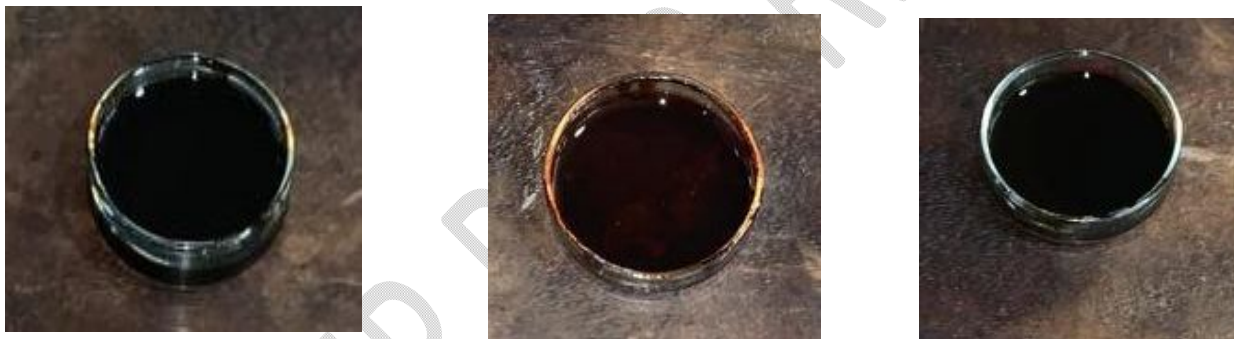
3. RESULTS

3.1 The Physicochemical Characteristics of the *H. iryaghedhi* Extracts

3.1.1 Nature of the Extracts

3.1.1.1 Nature of the Methanol Extracts

Methanol leaf extract was a dark green color and methanol bark extract and combination extract were dark brown color. Both extracts are sludge and have no odor.



(a)(b)

(c)

Figure 3: (a) Methanolic leaf extract, (b) Methanolic bark extract, (c) Methanolic combination extract of *Horsfieldia iryaghedhi*

3.1.1.2. Nature of aqueous extracts

Aqueous extracts were brown color crystals and had no odor.



Figure 4: (a) Aqueous leaf extract, (b) Aqueous bark extract, (c) Aqueous combination extracts of *Horsfieldia iryaghedhi*.

3.1.2 Yield of the Extracts

The yield of each extract was calculated by using the weight of each crude extract and the weight of the dry coarse powder of the *H.iryaghedhi* plant used for each extraction process.

3.2 The results of *in vitro* antibacterial activity of *Horsfieldiairyaghedhi*

3.2.1 *In vitro* antibacterial activity against *Staphylococcus aureus*

3.2.1.1 Antibacterial effect of Gentamycin (positive control) against *Staphylococcus aureus*

Table 1: Antibacterial effect of Gentamycin against *Staphylococcus aureus*

Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)
125	16.0 \pm 0.333
250	23.5 \pm 0.333
500	28.0 \pm 0.577
1000	31.5 \pm 0.667

According to Table 1, the highest antibacterial effect against *S. aureus* was observed with a concentration of 1000 $\mu\text{g/mL}$ while the lowest antibacterial effect was observed with a concentration of 125 $\mu\text{g/mL}$.

Dose- response curve of Gentamycin against *S. aureus*

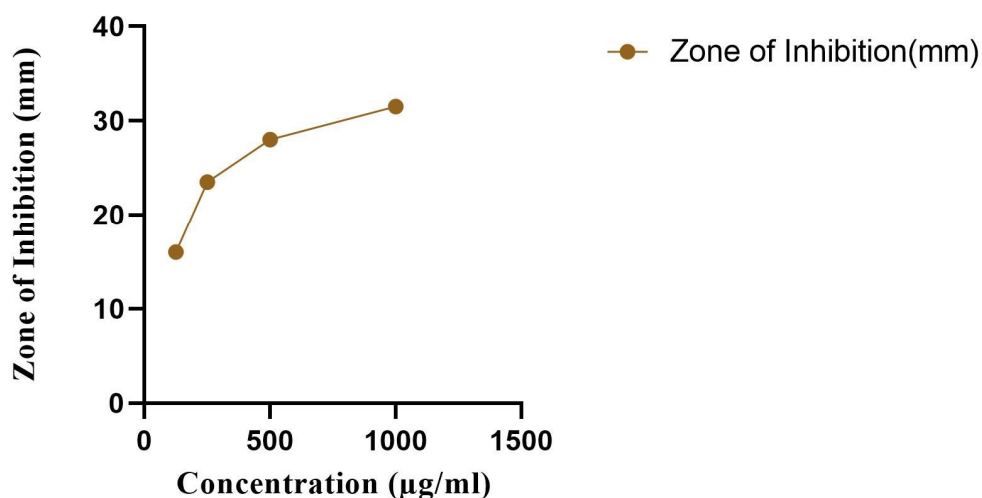


Figure 5: Dose-response curve of Gentamycin against *S.aureus*

Figure 6 shows that, with the increasing concentrations of Gentamycin, the zone of inhibition was also increased.

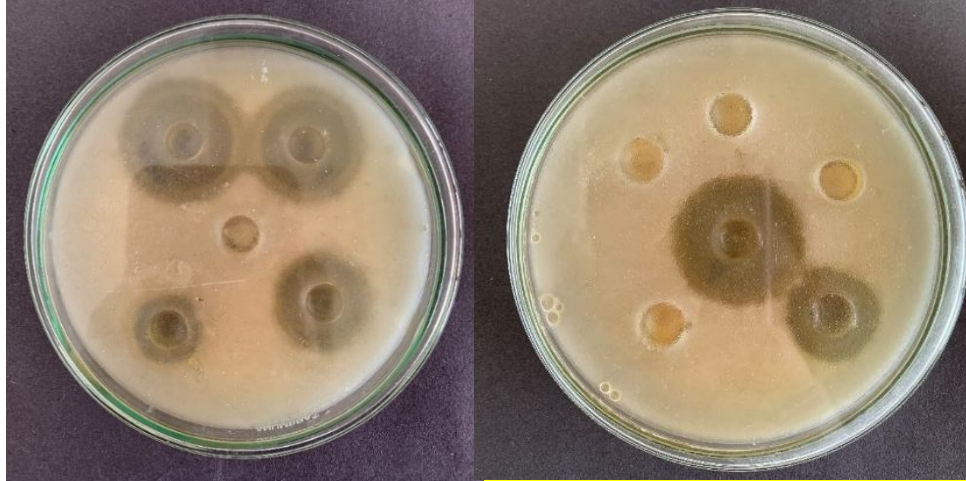


Figure 6: Effect of Gentamycin against *S.aureus* Figure 7: Effect of *H.horsfieldia* methanolic leaf extract against *S.aureus*

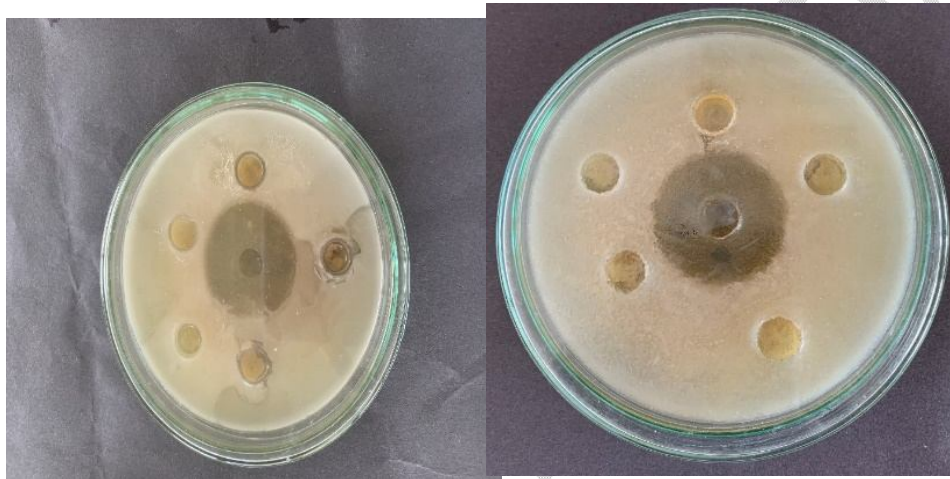


Figure 8: Effect of *H.iryaghedhi* methanolic extract against *S.aureus* Figure 9: Effect of *H.iryaghedhi* methanolic combination bark extract against *S.aureus*

3.2.1.2 Antibacterial effect of methanol extracts against *Staphylococcus aureus*

Table 2: Anti-bacterial effect of methanol extracts of *H.iryaghedhi* against *S. aureus*.

Concentration($\mu\text{g/mL}$)	Zone of inhibition (mm)		
	Leaf	Bark	Combination
125	-	-	-
250	-	-	-
500	12.6 \pm 0.667	-	12.32 \pm 0.667
1000	19.6 \pm 0.882	-	13.25 \pm 0.882

3.2.1.3 Anti-bacterial effect of aqueous leaf, bark, and combination extracts against *S.aureus*

Zone of inhibitions for leaf, bark, and combination aqueous extracts of *Horsfieldiaryghedhi* was not obtained against *S. aureus*.



Figure10: Effects of *H.iryaghedhi* aqueous extracts against *S.aureus*

3.2.2 In vitro antibacterial activity against *Escherichia coli*

3.2.2.1 Antibacterial effect of Gentamycin (positive control) against *E. coli*

Table 3: Antibacterial effect of Gentamycin against *E. coli*.

Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)
125	12.00 \pm 0.577
250	13.50 \pm 0.167
500	15.25 \pm 0.289
1000	25.00 \pm 0.333

According to Table 3, the highest antibacterial effect against *E. coli* was observed at the concentration of 1000 $\mu\text{g/mL}$ while the lowest antibacterial effect was observed at the concentration of 125 $\mu\text{g/mL}$.

Dose- response curve of Gentamycin against *E.coli*

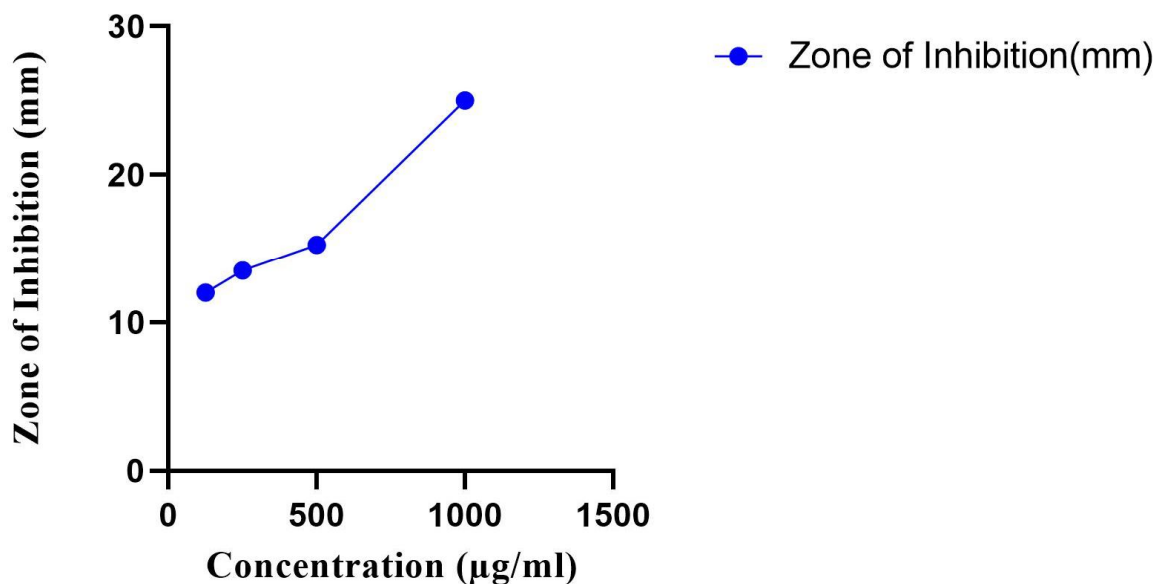


Figure 11: Dose-response curve of Gentamycin against *E.coli*

Figure 11 shows that with the increasing concentration of Gentamycin, the zone of inhibition was also increased.



Figure 12: Effect of Gentamycin against *E.coli*

3.2.2.2 Anti-bacterial effect of *H.iryaghedhi* extracts against *E. coli*

Zone of inhibition for leaf, bark, and combination of both methanol and aqueous extracts of *H. iryaghedhi* was not obtained against *E. coli*.

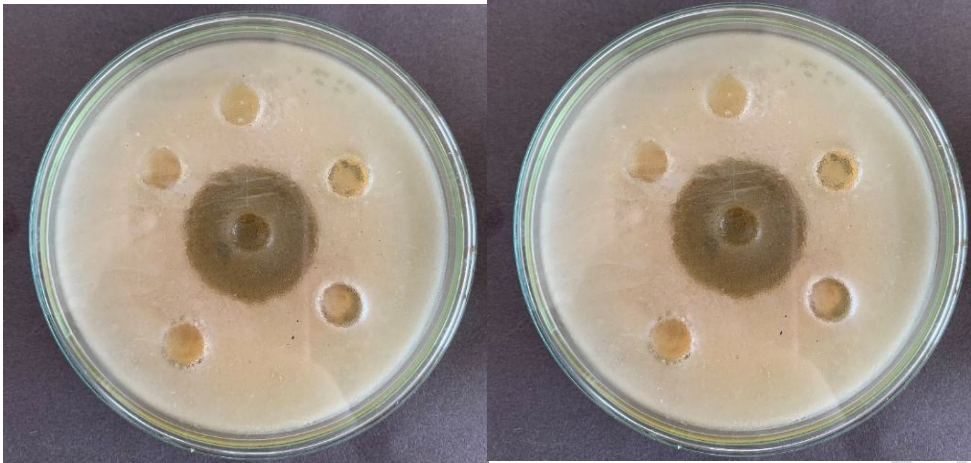


Figure 13: Effect of *H.iryaghedhi* methanol extracts against *E.coli*

Figure 14: Effect of *H.iryaghedhi* aqueous extracts against *E.coli*

3. DISCUSSION

The current need for the discovery of new and effective therapeutic medicines to treat and control various infectious diseases is pressing. In this context, Natural products have emerged as promising sources for innovative drug discovery, as they tend to have fewer side effects. This study aims to evaluate the antibacterial properties of different extracts of the medicinal plant *Horsfieldiaryaghedhi*.

Previous scientific studies and literature have reported the isolation of d-asarinin, dodecanol phloroglucinol, and dihydrocubebin from *Horsfieldiaryaghedhi* seeds. A detailed chemical investigation of the bark, leaf, and timber of *H. iryaghedhi* has resulted in the isolation of d-asaranin, dodecanol phloroglucinol, and dihydrocubebin. It was reported that dihydrocubebin, one of the primary chemical components, exhibited antimicrobial activity against *Mycobacterium smegmatis*(13).

Despite the plant's reported medicinal properties, the antibacterial activity of *Horsfieldiaryaghedhi* was yet to be established. Thus, this study aimed to evaluate the in vitro antibacterial activity of aqueous and methanol leaf, bark, and combination extracts of *H. iryaghedhi*.

In previous research, hot methanol was used to isolate the aforementioned chemical constituents. However, hexane was also used as a solvent for extraction, but no noticeable yield was obtained. In vitro, the antibacterial activity of the leaves, bark, and combination extracts was tested against gram-negative *E. coli* (ATCC® 25922) and gram-positive bacteria *Staphylococcus aureus* (ATCC® 25923)(13).

The well-diffusion method and disc diffusion method were considered acceptable methods of conducting antimicrobial assays. However, the exact quantity absorbed into the disc could not be accurately estimated in the disc-diffusion method, and contamination was possible.

The Myristicaceae family comprises over 80 species of the genus *Horsfieldia*, and most plants are widely used as antimicrobial agents in cosmetics and dermatological treatments. The healing properties of medicinal plants are mainly due to the presence of various secondary metabolites such as lignans, flavons, sterols, alkaloids, and essential oils (14).

For the present study, Gentamycin was selected as the positive control. The antibacterial activity of the positive control was tested against *Staphylococcus aureus* and exhibited a zone of inhibition of 31.5mm,

28mm, 23.5mm, and 16mm at concentrations of 1000µg/mL, 500µg/mL, 250µg/mL, and 125µg/mL, respectively.

The methanol leaf extract of *H. iryaghedhi* exhibited antibacterial activity against *S. aureus* for concentrations of 1000µg/mL and 500µg/mL, with the zone of inhibition being 19.6mm and 12.6mm, respectively. The methanol combination extracts of *H. iryaghedhi* exhibited a zone of inhibition of 13.25mm and 12.32mm for concentrations of 1000 µg/mL and 500 µg/mL, respectively. The r2 value of the reference drug Gentamycin was 0.9987 (p<0.05), and an IC50 value of 73.32mm was obtained.

In contrast, no antibacterial activity against *E. coli* was observed in any of the extracts tested. However, the positive control (Gentamycin) exhibited good antibacterial activity against *E. coli* with zones of inhibition of 25mm, 15.25mm, 13.50mm, and 12mm at concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL, respectively.

The results of the study showed that the methanol leaf and combination extracts of *Horsfieldiaryaghedhi* demonstrated a positive antibacterial response at the highest concentrations tested against gram-positive bacteria *Staphylococcus aureus* (ATCC® 25923). Additionally, the study's findings indicated that *H. iryaghedhi* plant extracts showed no antibacterial activity against gram-negative bacteria, *E. coli*, at the experimented concentrations.

As described in antibacterial studies, the secondary metabolites present in the plant are the primary reason for their medicinal properties. A high percentage of trimyristin (90%), the presence of myristic acid (0.00003%), d-asaranin (0.00014%), sitosterol (0.00001%), (-)-dihydrocubebin (0.000004%), and horsfieldin (0.0003%) must have contributed to the significant positive response of *Horsfieldiaryaghedhi* towards its antibacterial activity (13).

4. CONCLUSION

The present research study highlights the antibacterial activity of bark, leaf, and a combination of extracts of *Horsfieldiaryaghedhi* against gram-positive bacteria *Staphylococcus aureus* (ATCC® 25923) at their highest concentrations. However, the experimented concentrations of *H. iryaghedhi* plant extracts showed no antibacterial activity against gram-negative bacteria, *E. coli*. Notably, the methanol extracts of *Horsfieldiaryaghedhi*, an endemic plant in Sri Lanka, exhibited in vitro bacterial activity, as per the study results. This novel finding presents an opportunity for further research on the therapeutic potential of the *H. iryaghedhi* plant extracts..

REFERENCES

1. Sandle T. Firing the arrow with new antibiotics. 2021 Feb;6–8.
2. Jackson N, Czaplowski L, Piddock LJV. Discovery and development of new antibacterial drugs: Learning from experience? Vol. 73, Journal of Antimicrobial Chemotherapy. 2018. p. 1452–9.
3. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005 Aug;100(1–2):80–4.
4. Fitzgerald M, Heinrich M, Booker A. Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques. Front Pharmacol. 2019;10:1480.
5. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules. 2016 Apr;21(5).
6. Jayaweera DMA. Medicinal plants (indigenous and exotic) used in Ceylon. TA - TT -. Colombo SE - volumes <1-5> : illustrations (some color) ; 26 cm: National Science Council of Sri Lanka Colombo;
7. Senadeera SPNN, Fernando KSK, Wickramasekara WLLN, Fernando MYS, Ranaweera CB,

- Rajapaksha W, et al. Antibacterial Activity of Endemic *Artocarpus nobilis* Thw Found in Sri Lanka. *South Asian J Res Microbiol*. 2021;11(3):35–43.
8. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *J Pharm Bioallied Sci*. 2020;12(1):1–10.
 9. Haghgoo R, Mehran M, Afshari E, Zadeh HF, Ahmadvand M. Antibacterial Effects of Different Concentrations of *Althaea officinalis* Root Extract versus 0.2% Chlorhexidine and Penicillin on *Streptococcus mutans* and *Lactobacillus* (In vitro). *J Int Soc Prev Community Dent*. 2017;7(4):180–5.
 10. Silva A, Dissanayake R, Ranaweera C, Pathirana R, Ratnasooriya W. Evaluation Of In Vitro Antibacterial Activity of Some Sri Lankan Medicinal Plants. *Int J Pharm Res Allied Sci*. 2015 Jan;4:54–7.
 11. Moradian H, Bazargani A, Rafiee A, Nazarialam A. In vitro comparison of antimicrobial activity of aqueous decoction of *Coriandrum sativum*, and Dentol Drop with chlorhexidine on *Streptococcus mutans*. *Iran J Microbiol*. 2013 Sep;5(3):239–43.
 12. Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the Antibacterial and Antifungal Properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques. Filho VC, editor. *Sci World J [Internet]*. 2017;2017:9658598. Available from: <https://doi.org/10.1155/2017/9658598>
 13. Tillekeratne LM V, Jayamanne DT, Weerasuria KD V, Gunatilaka AAL. Lignans of *Horsfieldia iryagedhi*. *Phytochemistry [Internet]*. 1982;21(2):476–8. Available from: <https://www.sciencedirect.com/science/article/pii/S0031942200952993>
 14. Khayyat S, Alkattan M, Basudan N. Phytochemical Screening and Antidermatophytic Activity of Lavender Essential Oil from Saudi Arabia. *Int J Pharmacol*. 2018 Jul 15;14:802–10.