LIFE: International Journal of Health and Life-Sciences
ISSN 2454-5872

Samad & Silva, 2021

Volume 6 Issue 3, pp. 35-53

Date of Publication: 23rd January 2021

DOI- https://dx.doi.org/10.20319/lijhls.2021.63.3553

This paper can be cited as: Samad, S. A., & Silva, W. S. (2021). Phytochemical Analysis and Antibacterial Efficacy of Extracts of Dipterocarpus Zeylanicus. LIFE: International Journal of Health and Life-Sciences, 6(3), 35-53.

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EFFICACY OF EXTRACTS OF *DIPTEROCARPUS ZEYLANICUS*

Shamaa Abdul Samad
Faculty of Science, Department of Biotechnology, Horizon Campus, Malabe, Sri Lanka
shaamasamad@gmail.com

W. Sadin de Silva
Faculty of Science, Department of Biotechnology, Horizon Campus, Malabe, Sri Lanka
sadin@horizoncampus.edu.lk

Abstract

The overuse of antibiotics has resulted in microorganisms developing resistance to commercially available antibiotics. The present research aims to study the presence, properties, and distribution of bioactive compounds within several plant parts of Dipterocarpus zeylanicus which has been used in traditional medicine to treat various infectious diseases. The secondary metabolites were extracted from the leaf, seed, heartwood, and resin through sequential extraction. A qualitative phytochemical investigation was performed to determine the presence of secondary metabolites in the extracts. Antioxidant activity was determined using DPPH radical scavenging assay and Folin-Ciocalteu assay was used to measure the Total polyphenolic content (TPC). The antimicrobial assay was performed using EUCAST disc diffusion assay with Escherichia coli, MRSA, and Staphylococcus aureus as microbial strains. The phytochemical study indicated ubieties of alkaloids, steroids, saponins, flavonoids, cardiac glycosides, phenols, tannins, and terpenoids. Heartwood extracted using ethyl acetate showed the highest antioxidant activity (IC_{50} 0.484 µg/ml).

The highest amount of phenols (56.3±4.6 mg GAE/g) was present in the methanol extract of seed. A large inhibition zone (10.7±0.6 mm) by ethyl acetate extract of seed against E. coli demonstrated effective antibacterial activity. Therefore, these crude extracts can be used to isolate novel biologically active secondary metabolites exhibiting antimicrobial and antioxidant properties.

Keywords

Dipterocarpus Zeylanicus, Antioxidant Activity, Total Phenolic Content, Secondary Metabolites, $IC_{50,}$ Antimicrobial Activity

1. Introduction

Since the discovery of the first antibiotic, research has been done to find new antibiotics effective against a variety of pathogens.

1.1 Plant-Based Antibiotics

Treatment from plant extracts gained popularity in the early 1900s, since then plants have been recognized as an inexhaustible alternative source for the commercially available antibiotics for various diseases. Secondary metabolites produced in response to environmental stressors such as bacteria, viruses, and fungi even though not necessary for plant growth and development, play an indispensable role in the survival of the plant. Khameneh, Iranshahy, Soheili & Fazly Bazzaz, 2019 reported that some of these compounds while not effective themselves as antibiotics when used in conjunction with another antibiotic, can be used to overcome antibiotic resistance. Bacteria are evolving with new antibiotic-resistant mechanisms rendering the available drugs ineffective to treat common infectious diseases ("Antibiotic resistance", 2020).

1.2 Dipterocarpus Zeylanicus

Dipterocarpus Zeylanicus, renowned in Sinhalese as 'hora', is an endemic tall canopy tree that is found in the lowland evergreen forests of Sri Lanka (Attygalle & Singhakumara, 2013). Due to its excessive usage in commercial logging, *D. zeylanicus* has been classified as 'Endangered' in the IUCN Red List of Threatened Species (Ashton P., 1998).

Table 1: Taxonomy of *Dipterocarpus Zeylanicus* (Ashton P., 1998)

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	**Tracheophyta**	**Magnoliopsida**	**Theales**	**Dipterocarpaceae**	***Dipterocarpus***	***zeylanicus***

1.3 Medicinal Properties of *D. Zeylanicus*

Bandaranayake, Gunasekera, & Karunanayake, 1974 reported isolation of Asiatic acid from the resin of *D. zeylanicus* which is a saponin, a secondary metabolite with anti-diabetic and anti-

inflammatory properties. The heartwood and resin of *D. zeylanicus* was reported to have been used to treat various infectious diseases such as bronchitis and pneumonia ("Ayurvedic Plants of Sri Lanka: Plants Details", 2020). Weerasinghe & Deraniyagala, 2016 reported antioxidant activity of *D. zeylanicus* heartwood with a radical scavenging activity of 91.1±1% and a phenolic content of 64.4±2.1 mg PGE/g.

D. zeylanicus was selected for the present research due to its use in traditional medicine and the lack of research done on the antimicrobial activity of the plant metabolites.

2. Methodology

The procedures used in the present study are described below.

2.1 Sample Collection and Identification

Leaves, seeds, resin, and heartwood of *D. zeylanicus* were collected from Hayley's Horticulture Institute, Sri Lanka. A voucher specimen was submitted to Bandaranaike Memorial Ayurvedic Research Institute and was identified as *Dipterocarpus zeylanicus* (ACC no: 2035a).

2.2 Solvent Extraction

The collected plant parts (leaves, seeds, resin, and heartwood) were washed, air-dried, macerated, and placed in an airtight bag until further use. Sequential extraction was used to obtain the extracts from the plant parts of *D. zeylanicus* using hexane, ethyl acetate, methanol, and ethanol as industrial solvents.

2.3 Determination of Percentage Yield

The mass of the plant specimen and the resulting crude extract was used to measure the percentage yield, applying the equation,

$$\text{Percentage Yield } (\%) = \frac{W_2 - W_1}{W_0} \times 100 \qquad \textbf{(1)}$$

where W_2 is the mass of the extract and flask, W_1 is the mass of the flask and W_0 is the mass of the original dried plant specimen (Anokwuru, Anyasor, Ajibaye, Fakoya, & Okebugwu, 2011).

2.4 Phytochemical Analysis

A qualitative phytochemical study was performed for the *D. zeylanicus* crude extracts as described by Madike, Takaidza, & Pillay, 2017 to test for steroids, saponins, alkaloids, protein, anthocyanin, flavonoids, glycosides, phlobatannins, terpenoids, phenols, tannins, anthraquinones and carbohydrates. The formation of precipitate and the intensity of the color change was used as a measure of the analyses.

2.5 Percentage Radical Scavenging Activity (RSA)

The RSA of the crude extracts were analyzed by measuring the depreciating absorbance of the free radical, DPPH (1,1-diphenyl-2-picrylhydrazine) by a UV spectrophotometer at 517nm. The procedure was performed as described by Prieto, 2012.

Various concentrations of the crude extracts (500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.91, 1.95 µg/ml) were prepared using 90% methanol. About 100 µl extract was added to the wells of a 96 well plate, followed by 100 µl DPPH solution (1.0 mM) in methanol. About 100 µl of methanol in DPPH was used as blank. L- ascorbic acid was used as the reference standard. The well plate was placed in a dark room for twenty minutes and the absorbance was measured. (Marinova & Batchvarov, 2011). Percentage RSA was computed using the formula.

$$(\%)\ \text{RSA} = \frac{\mathrm{A}_0 - (A_1 - \mathrm{A}_2)}{A_0} \times 100 \qquad (\mathbf{2})$$

where, A_0 = absorbance of blank, A_1 = absorbance of the sample with DPPH, and A_2 = absorbance of sample without DPPH.

The half-maximal inhibitory concentration (IC_{50}) was calculated for Percentage RSA against log concentration using GraphPad™ Prism 8. The figures were recorded as mean values ± standard deviation.

2.6 Total Polyphenolic Content (TPC)

Polyphenolic compounds in the crude extracts of *D. zeylanicus* were assessed using Folin-Ciocalteau colorimetric method as outlined by Zhang, et al., 2006.

Different gallic acid concentrations (0.076 µg/ml to 2500 µg/ml) were prepared to obtain a calibration curve. The procedure used is described in Figure 1.

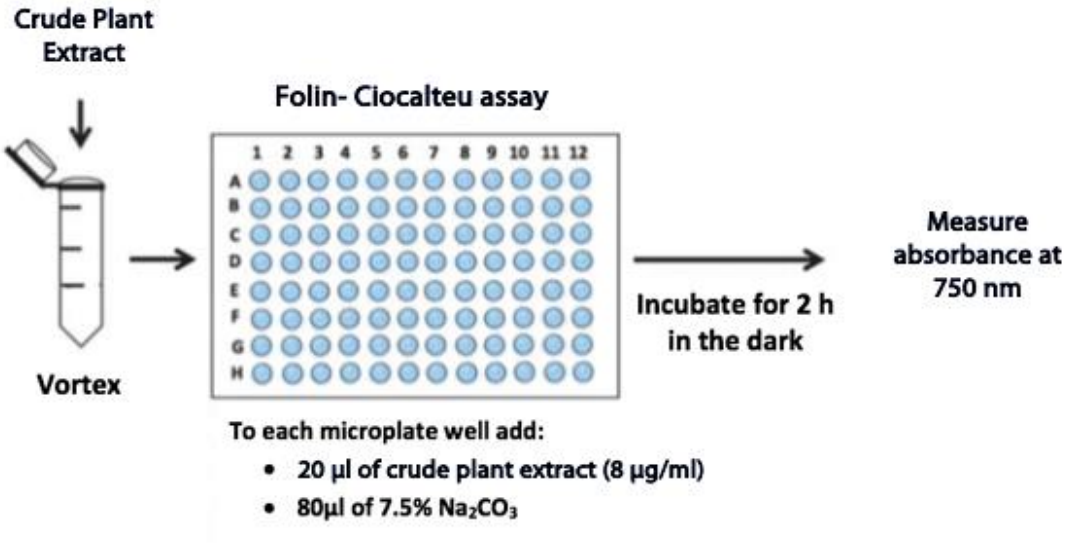

Figure 1: *Flow Chart Depicting the Folin-Ciocalteu Assay*

The results were recorded as mg gallic acid equivalents per gram of dry extract (mg GAE/g). TPC was computed using the equation,

$$C = \frac{c\,V}{m} \qquad (3)$$

where "C "denotes phenolic content in mg GAE/g, "c" represents gallic acid concentration obtained from the calibration curve in mg/mL, "V" is the volume and "m" is the mass of the extract.

2.7 Relationship between TPC and RSA

The correlation between TPC and RSA was established using GraphPad™ Prism 8, with TPC as mg GAE/g dry extract and RSA as IC_{50} values in µg/ml.

2.8 Antimicrobial Assay

The bacterial cultures susceptible to the crude plant extracts were investigated using EUCAST disc diffusion assay ("EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing", 2019). Test organisms used were *Escherichia coli ATCC® 25922™*, *Staphylococcus aureus ATCC® 25923™*, *and* Methicillin-resistant *Staphylococcus aureus* (MRSA) *ATCC® 43300™*. The turbidity of the inoculum was adjusted to 0.5 McFarland. About 500 µl of the inoculum was swabbed onto the surface of a Mueller-Hinton agar plate. Filter paper discs (6mm) impregnated with 400 µg/mL extract were transferred onto the agar plate and incubated overnight at 37°C. Gentamycin (20 μg/mL) was used as a positive control. The inhibition zone diameters were measured to the nearest millimeter. With the triplicate data, mean diameters were calculated and recorded as mean ± standard deviation.

3. Results

The results from the present study are given below.

3.1 Percentage Yield

Percentage yield obtained for each plant part using different solvents gave varying results. The highest yield was obtained for resin extracted using methanol (36.4%).

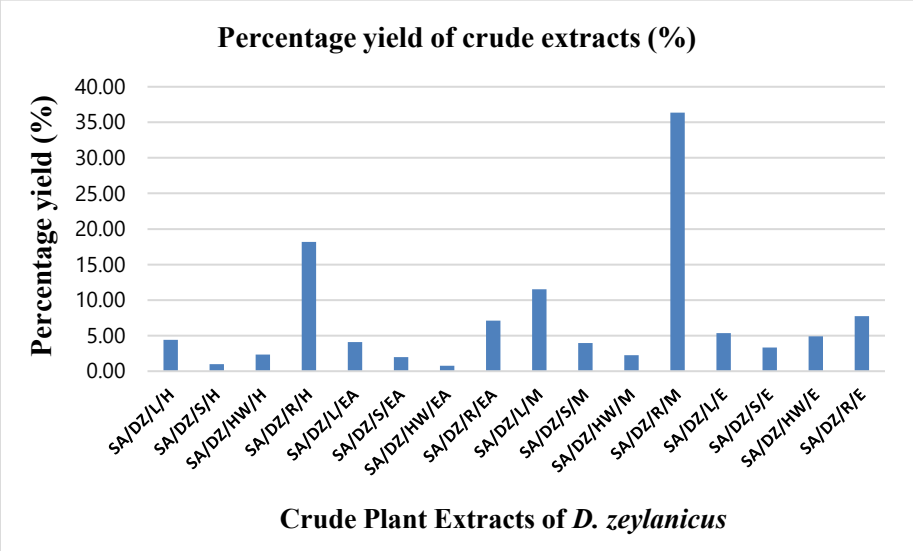

Figure 2: *Percentage Yield of Crude Plant Extracts of D. Zeylanicus*

3.2 Phytochemical Analysis

The phytochemical investigation acquainted the presence of saponins in all the crude fractions from hexane and ethyl acetate and the presence of alkaloids in all the extracts except methanol extract of the leaf. Most active constituents were present in the hexane, ethyl acetate, and methanol fraction of leaves with positive results for nine phytochemicals.

Table 2: *Results for the Phytochemical Analysis of Crude Extracts of D. Zeylanicus Leaves, Seeds, Heart Wood, and Resin*

Phytochemicals	Crude Plant Extracts															
	Hexane				Ethyl acetate				Methanol				Ethanol			
	L	S	H	R	L	S	H	R	L	S	H	R	L	S	H	R
Steroids	-	+	+	-	+	+	-	-	+	-	-	-	+	-	-	+
Saponins	+	+	+	++	+	+	++	+	-	-	-	+	-	-	-	+
Alkaloids	+	+	+	+	+	+	+	++	-	+	+	+	+	+	+	+
Protein	+	-	-	-	-	++	-	+	+	+	+	+	++	+	+	+
Anthocyanin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	-	+	+	+	+	+	+	-	+	+	++	-	+	+

Cardiac Glycosides	+	+	-	-	-	-	++	-	+	-	-	-	+	-	+	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoids	+	-	+	++	+	+	++	+++	+	-	+	+	+	+	+	-
Phenols	+	+	+	-	+	-	+	-	+++	+++	+++	++	+++	+++	+++	-
Tannins	+	+	+	-	+	-	+	-	+++	+++	+++	++	+++	+++	+++	-
Carbohydrate	+	+	-	-	+	+	-	-	+++	++	+++	+	++	+	+++	++
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key: **(L)** = Leaf, **(S)** = Seeds, **(H)** = Heartwood and **(R)** = Resin, **(+)** = Presence of active constituents, **(++)** = Present in moderate amounts, **(+++)** = Present in high amounts, **(−)** = Absence of active constituents.

3.3 Antioxidant Activity

The percentage of radical scavenging activity values were compared to the control, L-ascorbic acid to determine the strength of antioxidant activity. (± SD shows the mean of triplicate data)

3.3.1 Antioxidant Activity of Hexane Crude Extracts

A linear correlation was observed between the concentration and percentage radical scavenging values of resin, heartwood, and ascorbic acid, as the concentration increases, the percentage radical scavenging activity also increases. ANOVA test (Dunnett T3) showed the significant differences between the group means (Figure 4).

Table 3: *Percentage RSA of Hexane Extracts*

Concentration of extracts (µg/ml)	**DPPH Radical Scavenging Activity of Hexane Crude Extracts (%)**				
	Leaf	**Seed**	**Resin**	**Heartwood**	**Ascorbic Acid**
2.0	26.5 ± 5.7	27.8 ± 5.1	47.6 ± 2.8	33.7 ± 2.4	46.3 ± 1.4
3.9	26.5 ± 1.7	28.0 ± 3.6	47.7 ± 1.7	34.2 ± 1.4	49.9 ± 1.9
7.8	25.8 ± 1.8	27.8 ± 0.6	47.3 ± 2.6	35.2 ± 0.5	47.3 ± 4.7
15.6	27.0± 0.1	27.5 ± 2.7	47.8 ± 1.9	37.8 ± 2.3	49.3 ± 2.0
31.3	26.6 ± 4.1	28.6 ± 1.2	48.1 ± 3.4	40.3 ± 3.0	51.0 ± 3.3
62.5	26.8 ± 1.5	27.4 ± 3.6	49.2 ± 0.7	49.4 ± 6.1	51.9 ± 1.6
125.0	25.4 ± 2.2	25.8 ± 1.2	49.4 ± 3.7	64.7 ± 6.6	67.4 ± 1.2
250.0	26.0 ± 0.4	26.1 ± 8.5	50.6 ± 1.3	96.7 ± 3.4	90.0 ± 5.6
500.0	23.8 ± 1.8	24.4 ± 2.7	55.1 ± 3.0	98.2 ± 3.7	94.8 ± 4.1

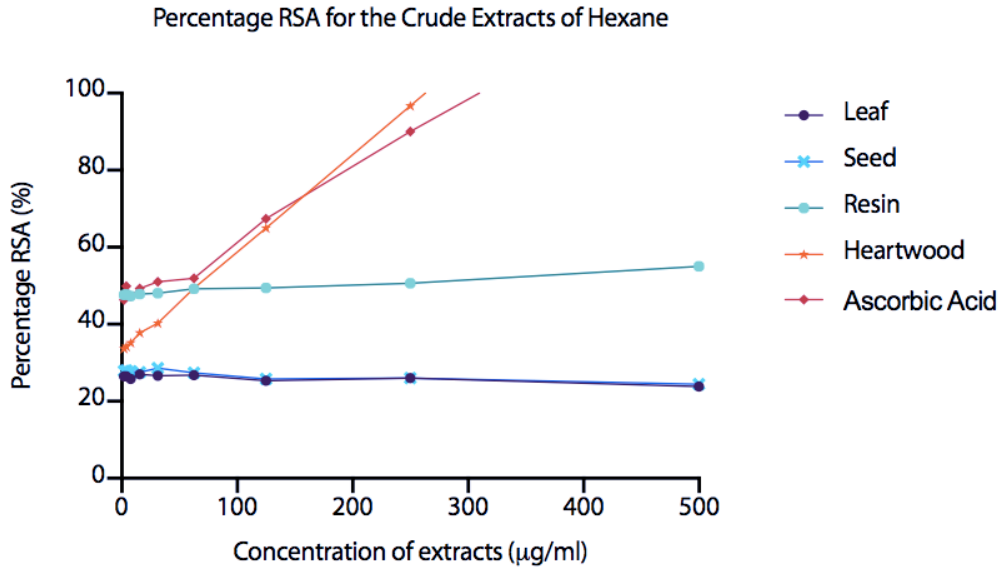

Figure 3: *Graph of Percentage RSA of Hexane Extracts*

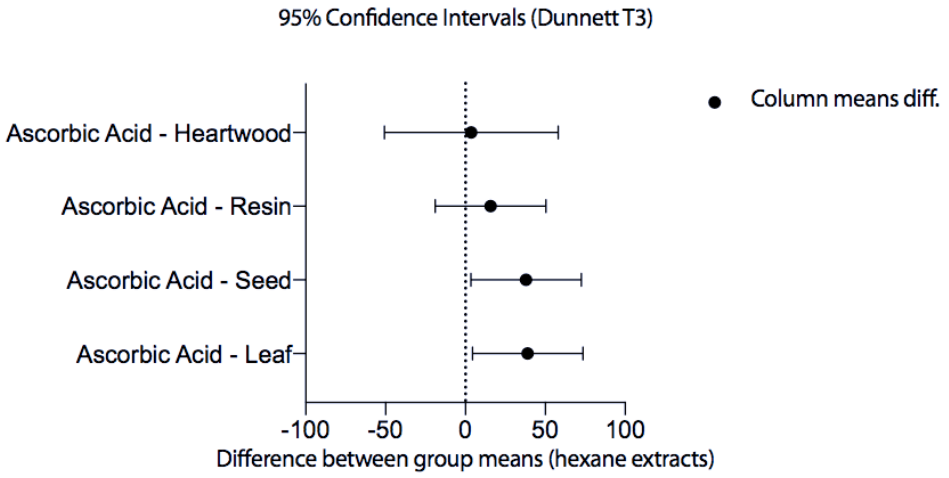

Figure 4: *ANOVA Statistical Test (Dunnett T3) of Hexane Extracts Against Ascorbic Acid*

3.3.2 Antioxidant Activity of Ethyl Acetate Extracts

As the concentration increases, for resin, heartwood, and ascorbic acid, the percentage of radical scavenging activity also increases. ANOVA test (Dunnett T3) showed the significant differences between the group means (Figure 6).

Table 4: *Percentage RSA of Ethyl Acetate Extracts*

Concentration of extracts (µg/ml)	DPPH Radical Scavenging Activity of Ethyl Acetate Crude Extracts (%)				
	Leaf	Seed	Resin	Heartwood	Ascorbic Acid
2.0	58.4 ± 6.8	41.0 ± 2.8	57.6 ± 3.7	52.3 ± 0.8	46.3 ± 1.4
3.9	59.6 ± 1.6	41.0 ± 8.1	57.8 ± 1.5	53.9 ± 2.9	49.9 ± 1.9
7.8	58.6 ± 3.8	41.0 ± 0.9	58.0 ± 3.5	53.6 ± 2.0	47.3 ± 4.7
15.6	56.4 ± 1.2	41.1 ± 1.1	58.6 ± 5.8	54.0 ± 3.3	49.3 ± 2.0
31.3	56.8 ± 0.3	40.9 ± 1.3	59.6 ± 1.6	54.2 ± 0.1	51.0 ± 3.3
62.5	55.7 ± 1.3	40.8 ± 2.7	57.5 ± 3.8	54.4 ± 2.9	51.9 ± 1.6
125.0	55.6 ± 4.4	40.3 ± 0.7	58.7 ± 2.6	55.1 ± 0.2	67.4 ± 1.2
250.0	44.1 ± 4.6	40.3 ± 5.8	74.2 ± 4.1	56.3 ± 1.1	90.0 ± 5.6
500.0	43.4 ± 4.3	39.9 ± 2.1	60.8 ± 6.3	60.6 ± 2.7	94.8 ± 4.1

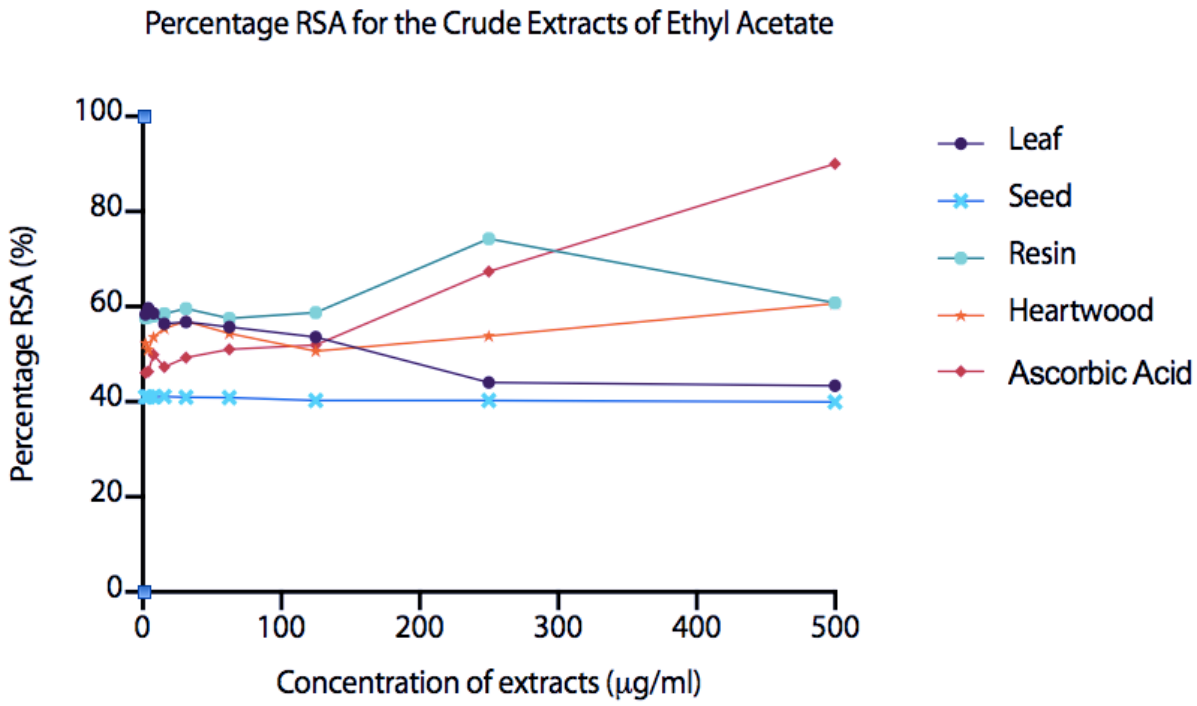

Figure 5: *Percentage RSA of Ethyl Acetate Extracts*

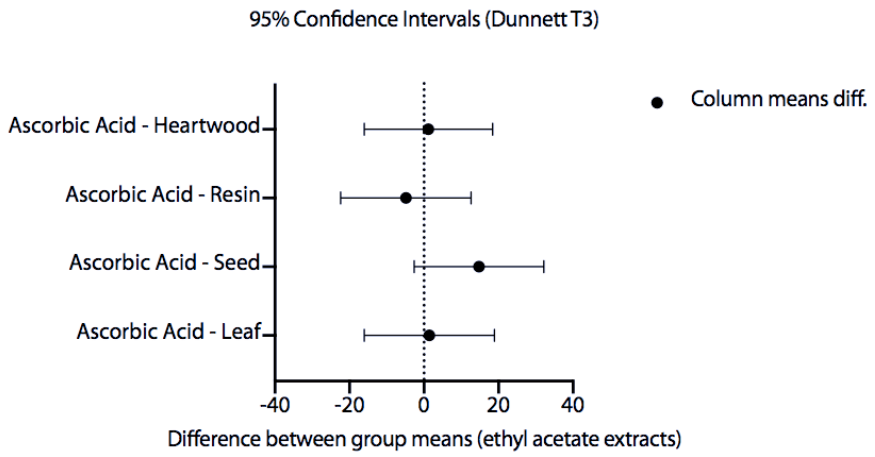

Figure 6: *ANOVA Statistical Test (Dunnett T3) of Ethyl Acetate Extracts Against Ascorbic Acid*

3.3.3 Antioxidant Activity of Methanol Crude Extracts

For leaves, resin, and heartwood, as the concentration increases the percentage of radical scavenging activity also increases. It was also observed that the resin extract had significantly higher antioxidant property in contrast to ascorbic acid. ANOVA test (Dunnett T3) showed the significant differences between the group means **(**Figure 8**)**.

Table 5: *Percentage RSA of Methanol Extracts*

Concentration of extracts (µg/ml)	**DPPH Radical Scavenging Activity of Methanol Crude Extracts (%)**				
	Leaf	**Seed**	**Resin**	**Heartwood**	**Ascorbic Acid**
1.953	49.7 ± 6.2	50.5 ± 1.6	54.8 ± 4.7	46.9 ± 3.9	46.3 ± 1.4
3.906	49.8 ± 2.9	50.9 ± 3.8	48.8 ± 4.9	46.9 ± 0.4	49.9 ± 1.9
7.813	49.9 ± 2.4	50.5 ± 3.3	50.3 ± 1.7	46.6 ± 4.2	47.3 ± 4.7
15.625	50.0 ± 1.2	49.9 ± 5.7	54.6 ± 5.7	47.1 ± 1.0	49.3 ± 2.0
31.250	50.3 ± 5.3	52.2 ± 3.7	64.7 ± 1.7	47.3 ± 2.9	51.0 ± 3.3
62.500	51.3 ± 3.8	52.3 ± 2.6	63.7 ± 2.8	48.2 ± 1.5	51.9 ± 1.6
125.000	52.1 ± 2.9	46.0 ± 7.1	72.8 ± 2.3	48.7 ± 1.6	67.4 ± 1.2
250.000	53.9 ± 2.6	48.7 ± 3.6	87.9 ± 2.0	50.4 ± 2.1	90.0 ± 5.6
500.000	59.3 ± 1.2	45.4 ± 3.2	88.9 ± 5.9	54.2 ± 5.6	94.8 ± 4.1

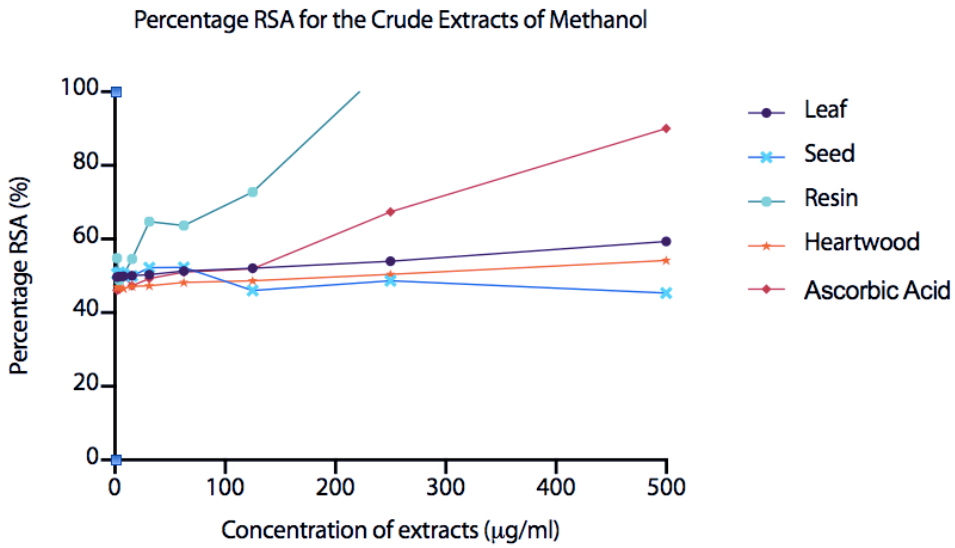

Figure 7: *Percentage RSA of Methanol Extracts*

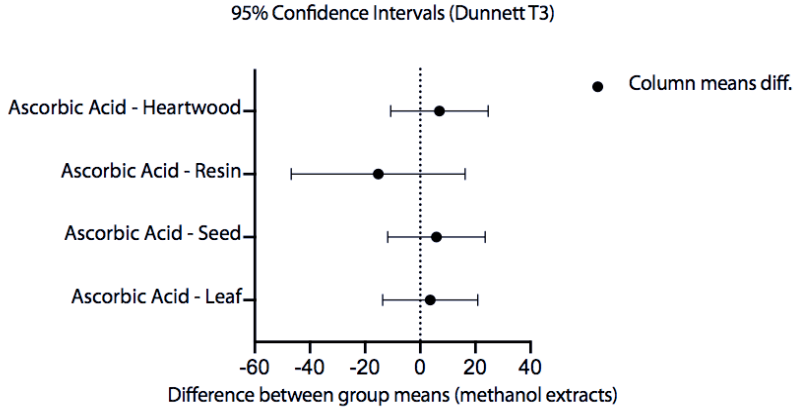

Figure 8. *ANOVA Statistical Test (Dunnett T3) of Methanol Extracts Against Ascorbic Acid*

3.3.4 Antioxidant Activity of Ethanol Crude Extracts

At lower concentrations, seeds and leaves showed higher antioxidant activity in comparison to L-ascorbic acid. However, at higher concentrations, leaves, and seeds exhibited lower antioxidant activity. ANOVA test (Dunnett T3) showed the significant differences between the group means (Figure 10).

Table 6: *Percentage RSA of Ethanol Extracts*

Concentration of extracts (µg/ml)	**DPPH Radical Scavenging Activity of Ethanol Crude Extracts (%)**				
	Leaf	**Seed**	**Resin**	**Heartwood**	**Ascorbic Acid**
2.0	57.8 ± 2.9	57.6 ± 4.8	41.6 ± 0.9	45.8 ± 1.1	46.3 ± 1.4
3.9	59.1 ± 1.8	57.7 ± 2.1	41.7 ± 5.8	45.8 ± 2.5	49.9 ± 1.9
7.8	61.1 ± 5.6	57.9 ± 6.6	41.8 ± 4.7	45.9 ± 1.5	47.3 ± 4.7

15.6	59.3 ± 2.6	58.2 ± 2.3	42.0 ± 2.2	45.9 ± 5.8	49.3 ± 2.0
31.3	61.7 ± 7.1	59.9 ± 1.3	42.3 ± 2.6	46.0 ± 2.2	51.0 ± 3.3
62.5	58.2 ± 5.0	58.8 ± 3.4	43.9 ± 3.5	45.4 ± 2.2	51.9 ± 1.6
125.0	59.5 ± 4.8	63.7 ± 0.2	44.0 ± 4.8	46.4 ± 3.2	67.4 ± 1.2
250.0	62.5 ± 5.6	68.6 ± 6.7	46.1 ± 3.7	49.1 ± 2.8	90.0 ± 5.6
500.0	65.8 ± 3.7	79.8 ± 1.5	53.3 ± 2.0	48.3 ± 3.3	94.8 ± 4.1

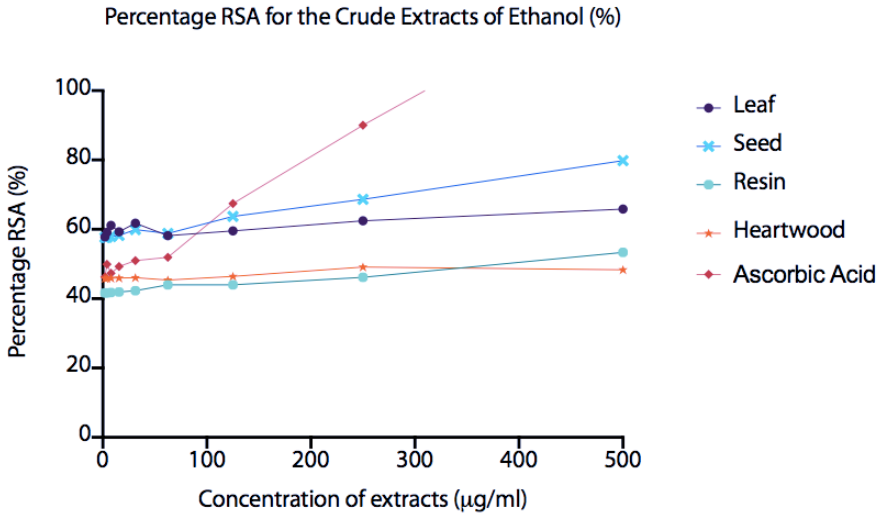

Figure 9: *Percentage RSA of Ethanol Extracts*

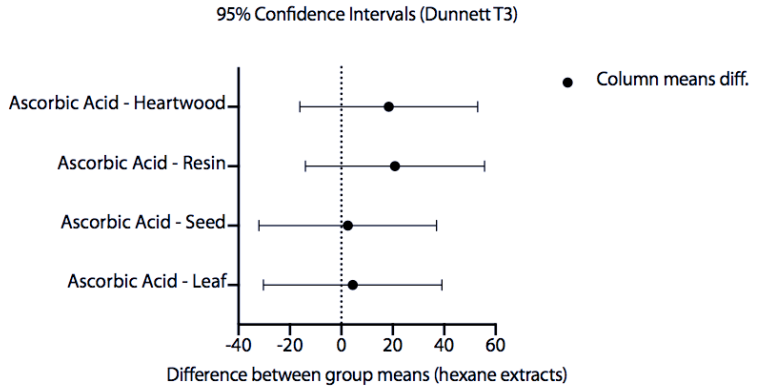

Figure 10: *ANOVA Statistical Test (Dunnett T3) of Methanol Extracts Against Ascorbic Acid*

3.3.5 Inhibitory Concentration (IC_{50})

The maximal Inhibitory Concentration (IC_{50}) was calculated using GraphPad Prism, non-linear regression. Ethyl acetate extract of heartwood, methanol extract of the leaf, and resin showed IC_{50} lower than that of ascorbic acid which was 12.01µg/ml.

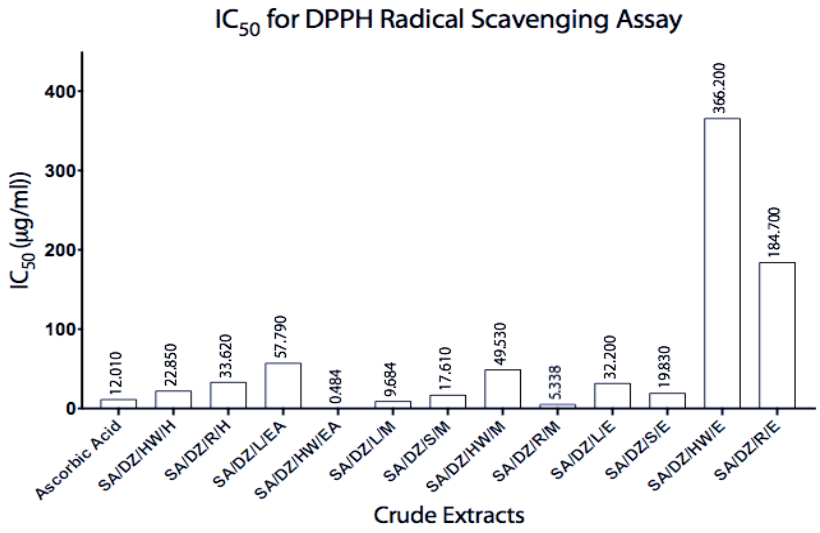

Figure 11: *IC_{50} Values for the Extracts of D. zeylanicus*

3.4 Total Polyphenolic Content (TPC)

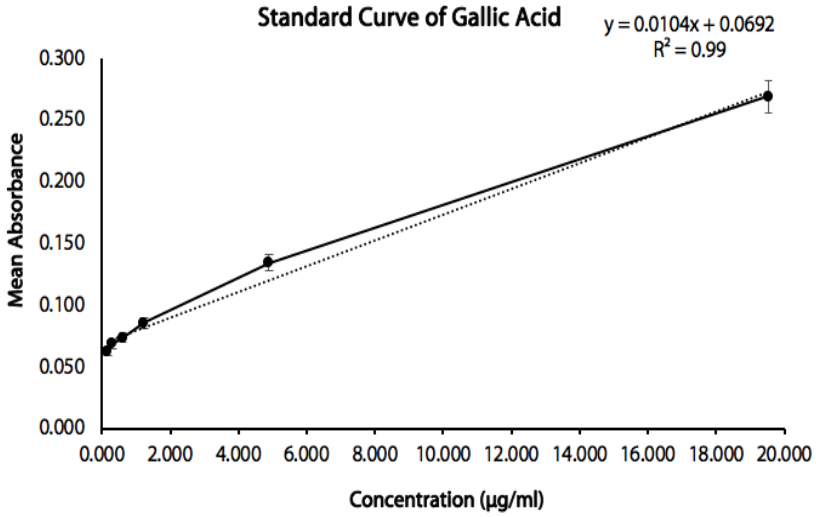

Figure 12: *Gallic Acid Standard Curve*

The absorbance values were used to calculate the GAE mg/g using the linear equation of the graph. The largest concentration of phenols was present in SA/DZ/S/M which contained 56.3 ± 4.6 mg GAE/g.

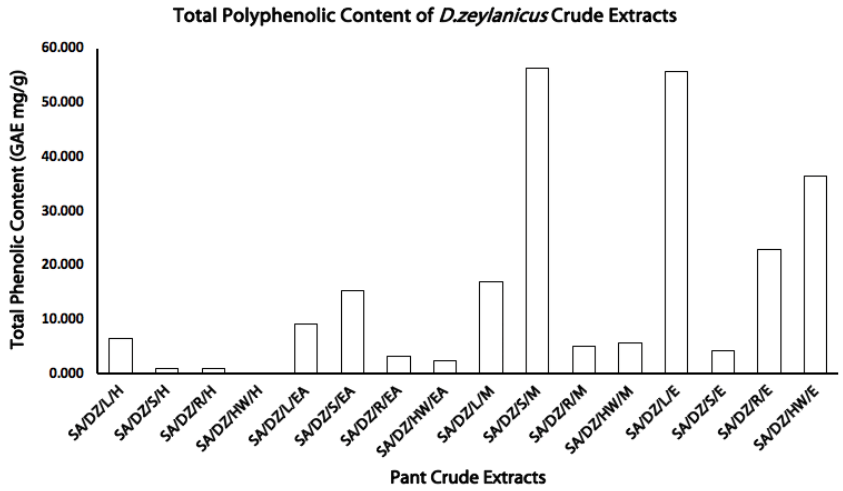

Figure 13: *TPC in the Extracts of D. Zeylanicus*

3.5 Antioxidant Activity and the Total Polyphenolic Content

The correlation between antioxidant activity and TPC is represented in the graph below. A positive correlation between RSA as IC_{50} and TPC of the samples illustrated the radical scavenging property of the phenols.

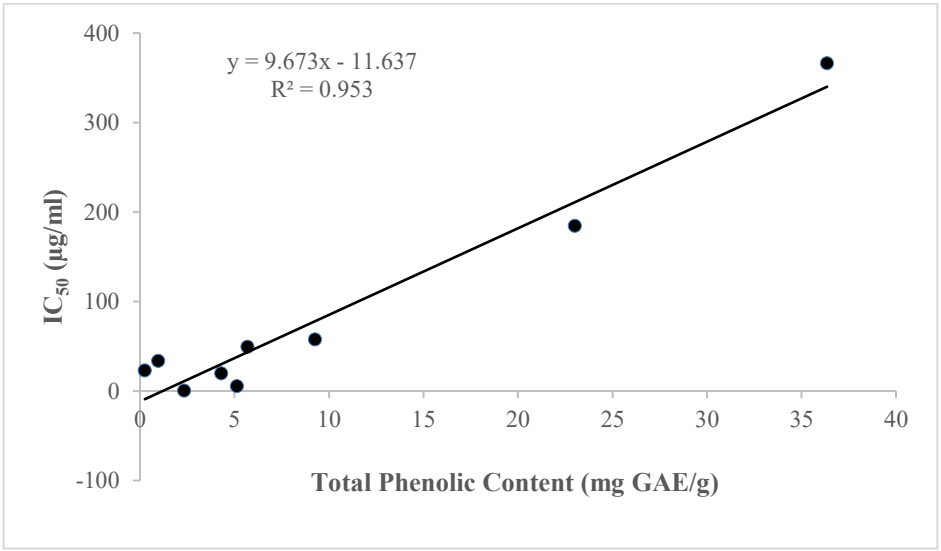

Figure 14: *Correlation between Antioxidant Activity and Total Polyphenolic Content*

3.6 Antimicrobial Assay

Ethyl acetate extract of seed (SA/DZ/S/EA) exhibited the highest inhibition zone (10.7±0.6 mm) against *E. coli*, followed by (SA/DZ/S/M) methanol extract of seed (10.3±1.0 mm) against *S. aureus*.

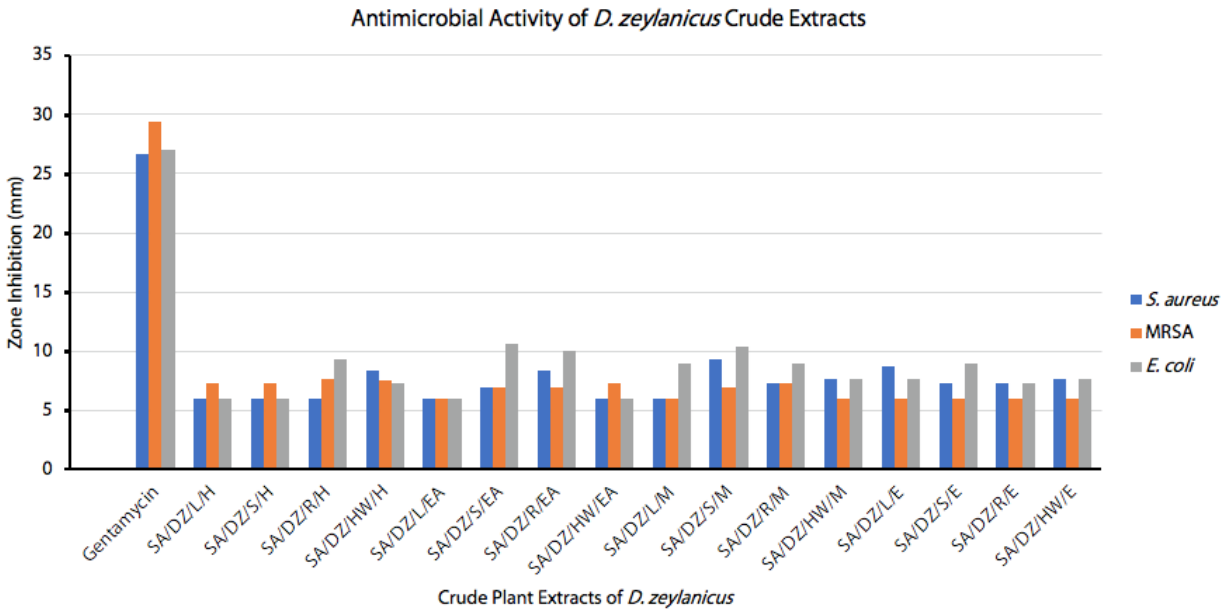

Figure 15: *Antimicrobial Activity of D. zeylanicus Crude Extracts at 400 µg/mL per Disc against selected Bacteria*

4. Discussion

The classification and analysis of bioactive compounds from plants are important to establish their medicinal properties (Madike, Takaidza, & Pillay, 2017). A broad range of plant metabolites is used to have commercial and industrial applications. The present study showed that pharmacologically active compounds such as saponins, alkaloids, steroids, protein, flavonoids, cardiac glycosides, terpenoids, phenols, tannins, and carbohydrates were found in organs of *D. zeylanicus* (Table 2). An interesting aspect of this investigation was that the hexane, ethyl acetate, and methanol fractions of leaves of the plant contained more phytochemicals than other plant parts. This may be due to the polarity of the compounds present in the extracts. (Senguttuvan, Paulsamy, & Karthika, 2014). Medicinal plants are being overexploited to the point of extinction. Since most secondary metabolites were present in the leaves of *D. zeylanicus*, the leaves can be collected, leaving the underground rhizome of the plant to regenerate itself. This has importance in conserving the species (Vikram, Chiruvella, Abdullah Ripain, & Arifullah, 2014). This is critical because *D. zeylanicus* is an endangered species that is endemic to Sri Lanka. Weerasinghe & Deraniyagala, 2016 reported antioxidant activity of *D. zeylanicus* heartwood with a radical scavenging activity of 91.1±1%. This finding goes in concert with the present study in that; phenols were qualitatively identified in the extracts of heartwood from both hexane and ethyl acetate (Table 2). Phenols exhibit

antioxidant properties. Furthermore, according to the Folin-Ciocalteau assay done by the same authors obtained a total phenolic of 64.4±2.1 mg as the total phenolic content of *D. zeylanicus* heartwood.

Half-maximal inhibitory concentration (IC_{50}) indicates the amount of drug needed to suppress a specific biochemical function by half (Akyul & Martinez-Hackert, 2016). Thus, the smaller the IC_{50} value, the smaller the amount of the extract required for activity. The IC_{50} value calculated for ascorbic acid was 12.01 µg/ml, so, the extracts having an IC_{50} value lower than that of ascorbic acid exhibits better antioxidant activity than ascorbic acid, which is considered a strong antioxidant (Figure 11). Ethyl acetate extract of heartwood (SA/DZ/HW/EA), seed, and resin extracted using methanol gave IC_{50} values, 0.484 µg/ml, 9.684 µg/ml, and 5.338 µg/ml respectively (Figure 11). This indicates that the amount of these extracts required to inhibit a reaction is less than ascorbic acid, making these extracts potent antioxidants. ANOVA statistical test proved the significant difference between the radical scavenging activity of different organs of *D. zeylanicus* when extracted with the same solvent. This suggests that the active compounds are distributed unevenly throughout the plant.

The extracts containing high concentrations of phenols exhibited high percentage of radical scavenging properties. The direct linear graph of IC_{50} against total polyphenolic content suggests the scavenging property is due to the presence of phenols (Figure 14). This is largely due to the redox potential of phenolic compounds, which can absorb and neutralize free radicals (S. M. Raquibul Hasan, et al., 2009). Weerasinghe & Deraniyagala, 2016 reported the phenolic content in the heartwood of *D. zeylanicus* extracted using methanol to be 64.4±2.1 mg PGE/g but the value found during the present study was 61.6±5.2 mg GAE/g (Figure 13). The values do not differ significantly, but the difference may be due to the concentration of DPPH or the concentration of extract.

The antimicrobial susceptibility was adopted to measure the potency of the extracts to be used as antimicrobial agents. Ethyl acetate extract of seed (SA/DZ/S/EA) gave the largest inhibition zone with 10.7±0.6 mm against *E. coli* (Figure 15). The phytochemical study of the same extract divulged the presence of several biologically active metabolites such as saponins which cause percolation of enzyme and protein from the cell (Mujeeb, Bajpai, & Pathak, 2014), flavonoids which are synthesized in plants in response to microbial infections (Sonam, Singh, & Pooja, 2017) and terpenoids which are responsible for disintegration of the cell wall of microorganisms (Mujeeb, Bajpai, & Pathak, 2014). The antimicrobial activity observed in the extract may be caused by the presence of these secondary metabolites.

During the present study, the compounds responsible for the elicited medicinal properties were not identified. For further studies HPLC can be adopted to determine the composition of the extracts.

5. Conclusion

The extracts of *D. zeylanicus* contained various biologically active compounds responsible for the antibiotic and antioxidant properties. These metabolites can be isolated and further studies can be performed to determine the pharmacokinetics and pharmacodynamics of the molecules exhibiting the medicinal properties in the present study. Khameneh, Iranshahy, Soheili & Fazly Bazzaz, 2019 reported that secondary metabolites can be co-administered with antibiotics in order to reduce the MIC values of antibiotics and to prevent antibiotic resistance. Further research can be done to determine whether these plant metabolites, in conjunction with commercially available antibiotics, are effective against antibiotic-resistant bacteria.

REFERENCES

Akyul, S., & Martinez-Hackert, E. (2016). Determination of half-maximal inhibitory concentration using biosensor-based protein interaction analysis. *Analytical Biochemistry*, 97-103. https://doi.org/10.1016/j.ab.2016.06.025

Anokwuru, C., Anyasor, G., Ajibaye, O., Fakoya, O., & Okebugwu, P. (2011). Effect of Extraction Solvents on Phenolic, Flavonoid and Antioxidant activities of Three Nigerian Medicinal Plants. *Nature and Science, 9*(7), 55.

Antibiotic Resistance. (2020). Retrieved 1 August 2020, from https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Ashton, P. (1998). IUCN Red List of Threatened Species: Dipterocarpus zeylanicus. Retrieved 1 March 2019, from https://doi.org/10.2305/IUCN.UK.1998.RLTS.T30807A9578732.en

Attygalle, P., & Singhakumara, B. (2013). THE ECOLOGY OF Dipterocarpus zeylanicus PLANTATION AT INGIRIYA, SRI LANKA. *Proceedings Of International Forestry And Environment Symposium*,. https://doi.org/10.31357/fesympo.v0i0.1426

Ayurvedic Plants of Sri Lanka: Plants Details. (2020). Retrieved 1 December 2020, from http://www.instituteofayurveda.org/plants/plants_detail.php?i=424&s=Scientific_name

Bandaranayake, W., Gunasekera, S., & Karunanayake, S. (1974, November 10). Terpenes of Dipterocarpus and Doona species. *Phytochemistry*, *14*(9), 2043-2048. https://doi.org/10.1016/0031-9422(75)83122-0

EUCAST. (2019). EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing. *Antimicrobial Susceptibility Testing*, pp. 5-15.

Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. (2019). Review on plant antimicrobials: a mechanistic viewpoint. Antimicrobial Resistance & Infection Control, 8(1), 23. https://doi.org/10.1186/s13756-019-0559-6

Madike, L. N., Takaidza, S., & Pillay, M. (2017). Preliminary Phytochemical Screening of Crude Extracts from the Leaves, Stems, and Roots of Tulbaghia violacea. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(10), 1300-1305. https://doi.org/10.25258/phyto.v9i10.10453

Marinova, G., & Batchvarov, V. (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. *Bulgarian Journal of Agricultural Science,*, *17*(1), 11-24.

Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of Aegle marmelos. *Biomed Research International*, *2014*, 3-9. https://doi.org/10.1155/2014/497606

Prieto, D. (2012). Dr Prieto's DPPH Microplate Protocol, 1-3.

Raquibul Hasan, S. M., Hossain, M., Akter, R., Jamila, M., Mazumder, E. H., & Rahman, S. (2009). DPPH free radical scavenging activity of some Bangladeshi medicinal plants. *Journal of Medicinal Plants Research*, 875-879.

Senguttuvan, J., Paulsamy, S., & Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pacific Journal of Tropical Biomedicine*, *4*(Suppl 1), 359-367. https://doi.org/10.12980/APJTB.4.2014C1030

Sonam, M., Singh, R. P., & Pooja, S. (2017). Phytochemical Screening and TLC Profiling of Various Extracts of Reinwardtia indica. *International Journal of Pharmacognosy and Phytochemical Research*, *9*(4), 523-527. https://doi.org/10.25258/phyto.v9i4.8125

Vikram, P., Chiruvella, K. K., Abdullah Ripain, I. H., & Arifullah, M. (2014, June). A recent review on phytochemical constituents and medicinal properties of kesum (Polygonum minus

Huds.). *Asian Pacific Journal of Tropical Biomedicine, 4*(6), 430-435. https://doi.org/10.12980/APJTB.4.2014C1255

Weerasinghe, W., & Deraniyagala, S. (2016). Antioxidant activity of some Sri Lankan Medicinal Plants. *Pharmaceutical Journal of Sri Lanka, 6*(1). https://doi.org/10.4038/pjsl.v6i0.10

Zhang, Q., Zhang, J., Shen, J., Silva, A., Dennis, D., & Barrow, C. (2006). A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. *Journal of Applied Phycology*, (18), 445-450. https://doi.org/10.1007/s10811-006-9048-4