

Antioxidant and sun screening activity of *Mangifera zeylanica* bark extracts

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Abstract *Mangifera zeylanica* is an indigenous plant in Sri Lanka with a rich history in traditional medicine. The bark of this plant, collected from two distinct regions of the country, has been explored in this study for its potential as a source of antioxidants and sun protection agents. Methanolic and aqueous extracts, and their separated fractions obtained from column chromatographic separation, were investigated. Phytochemical analysis of the extracts revealed the presence of flavonoids and polyphenols. Methanolic inner bark extracts collected from two regions and aqueous extract of inner bark collected from Rambukkana exhibited lower IC₅₀ values of 7.378, 17.56 and 16.82 µg/mL while the separated fraction 01 of methanolic inner bark extracts of Galle and Rambukkana have also shown lower IC₅₀ values of 11.71 and 13.00 µg/mL showing their potent antioxidant activity. Moreover, the methanolic inner bark extracts of Galle and Rambukkana exhibited a Sun Protection Factor (SPF) of 30.97 ± 0.11 and 29.88 ± 0.02 at the concentration of 1024 µg/mL. Among the separated fractions, fraction 01 of these extracts possessed the highest Sun Protection Factors of 30.19 ± 1.35 and 30.63 ± 0.19. The findings suggest that chemical compounds separated into fraction 01 are responsible for their sun protection activity and antioxidant activity. These findings underline the potential of fraction 01 methanolic extracts of *M. zeylanica* bark extracts as valuable sources of antioxidants and sun protection agents.

Keywords: Antioxidant, *Mangifera zeylanica*, Sun protection activity, Sun Protection Factor

Introduction

Medicinal plants have been an integral part of traditional healing practices for centuries that offer therapeutic benefits. These plants are endowed with a diverse array of bioactive compounds serving as reservoirs for the treatment of various ailments (Bhairam et al., 2013). Among these natural herbs, Mango (*Mangifera indica*), a member of the Anacardiaceae family, is popular for its delicious fruits as well as the medicinal value of different parts of the plant. The fruit, bark, seeds, and flowers of this plant have been used in traditional medicine for the treatment of diverse ailments (Khare, 2004; Ediriweera et al., 2016a). This rich history of employing mango for diabetes, asthma, gastric disorders, dysentery, and mouth sores underscores

its therapeutic significance (Ediriweera et al., 2016b). Studies have indicated antimicrobial, antioxidant, and anticancer activities, particularly in *M. indica*. However, there have been limited investigations into these properties on other mango species

Mangifera zeylanica is a wild mango species which is commonly known as 'Etamba'. It is an endemic plant to Sri Lanka which bears edible fruits, but it is not cultivated in the home gardens. This plant is typically found in intermediate and wet zone forests in Sri Lanka as a slow growing large tree (Weerarathne et al., 2005). The stem bark of *M. zeylanica* is used for its anti-inflammatory properties and anti-cancer properties (Ediriweera et al., 2016b). The present work was undertaken to analyze the antioxidant and sun screening

properties of the *M. zeylanica* stem bark extracted into hexane, methanol and water. Further the bioactive compounds with antioxidant and sun protection activity were separated through column chromatography and analyzed using GC-MS analysis.

Materials and methods

Plant material

M. zeylanica stem bark was collected from two randomly selected areas of the wet zone, Rambukkana divisional secretariat of Kegalle District 7° 18.821'N 80° 23.297'E and Neluwa divisional secretariat of Galle District 6° 22.408'N 80° 21.451'E. The plants were authenticated at Bandaranayaka Memorial Ayurvedic Research Institute, Nawinna, Maharagama and voucher

specimens were deposited in their collection (Acc No 3004).

Preparation of the extracts

The stem barks were separated into inner bark and the outer bark. They were washed separately with distilled water, subsequently air dried and coarsely powdered. Twenty grams (20 g) of the powdered bark from each sample was extracted with 200 mL of hexane, methanol and water by refluxing for 60 minutes separately and the resulting filtrate was obtained. The hexane and methanol extracts were rotary evaporated and water extract was freeze dried to obtain the solid crude extract. The percentage yield of the crude extracts was calculated and plant extracts with highest yield were selected for further analysis. Twelve extracts were prepared from the two plant specimens using hexane, methanol and water from outer and inner bark as shown in table.

Table 1: Plant extracts and the abbreviations used in the study

Name of the plant extract	Abbreviation used in the study
Methanol extract of Galle inner bark	GMI
Methanol extract of Galle outer bark	GMO
Methanol extract of Rambukkana inner bark	RMI
Methanol extract of Rambukkana outer bark	RMO
Water extract of Galle inner bark	GW
Water extract of Galle outer bark	GWO
Water extract of Rambukkana inner bark	RW
Water extract of Rambukkana Outer bark	RWO
Hexane extract of Galle inner bark	GHI
Hexane extract of Galle outer bark	GHO
Hexane extract of Rambukkana inner bark	RHI
Hexane extract of Rambukkana Outer bark	RHO

Phytochemical analysis of the plant extracts screening for alkaloids

An amount equivalent to 50 g of crude extract was measured and 10 mL of 2 M HCl was added. The mixture was heated for 3-5 minutes, then allowed to cool and mixed with 0.5 g of powdered NaCl. The mixture was filtered, and filtrate was separated into

three test tubes. Test tube 01 was used as the negative control. A few drops of Mayers' reagent were added into test tube 02 and few drops of Wagners' reagent were added into the test tube 03. The colour changes and precipitation formation were observed and compared with the negative control (Kancherla et al., 2019).

Screening for saponins

Fifty milligrams (50 mg) of dried plant extract was mixed with 5 mL of distilled water and shaken well for about 30 seconds. Then the test tube was allowed to stand in a vertical position and observed over for a 30-minute period. Development of a stable foam appearance showed the presence of saponins (Gul et al., 2017).

Screening for flavonoids and leucoanthocyanins

Plant extract sample (3 g) was measured and triturate with 15 mL of petroleum ether. The mixture was filtered, and the trituration of the residue was repeated with additional volume of petroleum ether until the petroleum ether phase became colourless. The residue was obtained and dissolved in 30 mL of 80% ethanol. The resulting mixture was filtered and separated into 3 test tubes each containing 1-2 mL of the filtrate. Test tube 01 was used as the negative control while test tube 02 was used for the cyanidin test and test tube 03 was used for the leucoanthocyanine test.

Cyanidin test

Conc. HCl 0.5 mL and 3 or 4 Magnesium turnings were added into test tube 02 and observed for the colour change. Appearance of pink colour indicate the presence of flavonoids (Kumar et al., 2012).

Test for leucoanthocyanine

Conc. HCl (0.5 mL) was added into test tube 03 and the resulting mixture was heated on a steam bath for 5 minutes. Change in the colour of the filtrate was observed and compared with the negative control. Turning the mixture into cherry red confirms the presence of leucoanthocyanine (Kumar et al., 2012).

Screening for Tannins and polyphenols

A plant extract of 10 g was mixed with 25 mL of hot distilled water. The mixture was allowed to cool

to room temperature and filtered. Then the filtrate was divided into 3 test tubes. Test tube 01 was used as the negative control. Test tube 02 was used for the gelatin test and test tube 03 was used for the ferric chloride test.

Gelatin test

To test tube 02, 4-5 drops of 1% gelatin salt solution (1% gelatin and 10% NaCl) was added and formation of precipitate indicated the presence of tannins and phenolic compounds (Kumar et al., 2012).

Ferric chloride test

To test tube 03, 4 – 5 drops of FeCl₃ was added. Formation of a dark green colour solution indicates the presence of polyphenols (Kumar et al., 2012; Mamta & Jayoti, 2012).

Antioxidant activity

Antioxidant activity was measured using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging assay using the method mentioned by Biswas *et al.* (2017)

DPPH radical scavenging assay

A double dilution series of 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1 µg/mL was prepared using the crude powder/ separated fractions in ethanol. Two hundred microliters (200 µL) of each dilution and 600 µL of 0.004% ethanolic DPPH solution was added together, shaken vigorously for 15 seconds and kept in the dark for 30 minutes to promote the reaction. The absorbance of the mixtures was measured at 517 nm and the percentage scavenging activity was calculated using the following equation. Ascorbic acid was used as the positive control.

$$\text{Percentage Scavenging} = \frac{[\text{Absorbance of the control} - \text{Absorbance of the test sample}]}{\text{Absorbance of the control}} \times 100\%$$

Sun protection activity assay

A stock solution of plant extract/separated fractions at a concentration of 1024 µg/mL was prepared in ethanol and filtered through Whatman No. 1 filter paper to obtain the filtrate. Subsequently, a double dilution series ranging from 512 to 1 µg/mL was

generated using the filtrate. The absorption of each extract was obtained in the range of 290 nm to 320 nm, every 5nm using 96 well plate reader (Thermoscientific Multiskan Sky) (Kaur & Saraf, 2017; Malsawmtluangi et al., 2013). The SPF of the samples was calculated using the Mansur equation (Mansur et al., 1986).

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where,

CF = correction factor (which is equal to 10)

EE (λ) = erythemogenic effect of radiation with wavelength λ

I = Solar intensity spectrum

Abs (λ) = spectrophotometric absorbance values at wavelength λ.

The values of EE x λ are constants, which were determined by (Sayre et al., 1979).

Table 2: Normalized product function used in the calculation of SPF

Wavelength (λ nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.000

The active fractions of *M. zeylanica* were selected and subjected to bioactivity guided fractionation.

Development of the Thin Layer Chromatography (TLC) profile

The TLC profile of the selected plant extracts was developed combining different solvent systems in different ratios to obtain the best resolution (Chloroform: Methanol: Hexane: Formic Acid 7:3:2:1). The developed TLC was observed under 254 nm wavelength fluorescence lamp and solvent front and the spots were determined. The Retention factor (Rf) value was calculated using the following formular.

$$\text{Retention factor (Rf)} = \frac{\text{Distance travelled by the plant extract}}{\text{Distance travelled by the solvent font}}$$

Column chromatographic separation

Using column chromatography, the extracts with the highest antioxidant and sun protection activities were fractionated. Serving ethyl acetate as the mobile phase and silica gel as the stationary phase, a column with a diameter of 15 mm and a length of 30 cm was filled. Extract (0.5g) was added as a slurry to the packed column. Various ethyl acetate and methanol ratios were used to elute the fractions. Eluents were collected at intervals of 10 mL, and

the obtained fractions were analyzed through TLC. The antioxidant and sun protection activity of the fractions with comparable profiles was assessed.

Results

The highest yield percentage was exhibited by the methanol and water extracts while the hexane extracts exhibited the lowest yield percentage (Table 3). Therefore, plant extracts of methanol and water were selected for further testing.

Table 3: Plant extracts with their percentage yields

Sample Name	Percentage Yield (%)
Rambukkana methanol inner bark (RMI)	8.54
Rambukkana methanol outer bark (RMO)	5.83
Rambukkana water inner bark (RWI)	3.75
Galle water outer bark (GWO)	3.15
Galle water inner bark (GWI)	2.99
Rambukkana water outer bark (RWO)	2.56
Galle methanol inner bark (GMI)	2.19
Galle methanol outer bark (GMO)	1.13
Galle hexane inner bark (GHI)	0.05
Galle hexane outer bark (GHO)	0.03
Rambukkana hexane inner bark (RHI)	0.02
Rambukkana hexane outer bark (RHO)	0.01

Phytochemical analysis of the plant extracts

Among the phytochemicals tested flavonoids and polyphenols gave a positive reaction for methanol and water extracts of plant (Table 4).

Table 4: Phytochemical evaluation of *M. zeylanica* bark extracts

Phytochemical Test	Results							
	GMI	GMO	GW	GWO	RMI	RMO	RWI	RWO
Test for alkaloids								
Mayer's reagent	-	-	-	-	-	-	-	-
Wagner's reagent	-	-	-	-	-	-	-	-
Test for saponins								
Froth test	-	-	-	-	-	-	-	-
Test for flavonoids								
Cyanidin test	+	+	+	+	+	+	+	+
Test for leucoanthocyanine								
Leucoanthocyanine test	-	-	-	-	-	-	-	-
Test for polyphenols and tannins								
Ferric chloride test	+	+	+	+	+	+	+	+
Gelatin test	-	-	-	-	-	-	-	-

Note - (+) Positive test results, (-) Negative test results

Antioxidant activity of the plant extracts

The highest percentage scavenging activity were shown by GMI, GWI and RMI extracts of $94.32 \pm 0.29\%$, $91.97 \pm 1.20\%$ and $91.97 \pm 1.20\%$ respectively at the concentration of $1024 \mu\text{g/mL}$

while the positive control, ascorbic acid exhibited a percentage scavenging activity of $96.30 \pm 0.13\%$ (Table 5).

Table 5: Percentage scavenging activity of the plant extracts and standard drug tested for antioxidant activity

Concentration ($\mu\text{g/ml}$)	Galle Methanol Inner bark (GMI)	Galle Methanol Outer bark (GMO)	Galle Inner (GWI)	Water bark	Galle Outer (GWO)	Water bark	Rambukkana Methanol Inner bark (RMI)	Rambukkana Methanol Outer bark (RMO)	Rambukkana Water Inner bark (RWI)	Rambukkana Water Outer bark (RWO)	Ascorbic acid Control (AA)
1024	94.32 $\pm$ 0.29	89.41 $\pm$ 1.39	91.97 $\pm$ 1.20		89.64 $\pm$ 1.72		91.97 $\pm$ 1.20	87.80 $\pm$ 1.11	84.73 $\pm$ 1.57	85.55 $\pm$ 1.53	96.45 $\pm$ 0.13
512	94.83 $\pm$ 0.11	91.20 $\pm$ 0.72	94.78 $\pm$ 0.52		82.98 $\pm$ 0.66		90.24 $\pm$ 0.16	65.01 $\pm$ 0.73	90.71 $\pm$ 1.41	64.32 $\pm$ 0.61	96.42 $\pm$ 0.11
256	95.07 $\pm$ 0.04	92.05 $\pm$ 1.82		74.71 $\pm$ 0.69	76.31 $\pm$ 0.38		91.98 $\pm$ 0.23	53.22 $\pm$ 0.22	94.50 $\pm$ 0.12	51.22 $\pm$ 1.54	95.77 $\pm$ 0.44
128	94.81 $\pm$ 0.42	73.20 $\pm$ 1.66		51.80 $\pm$ 1.50	62.24 $\pm$ 1.29		92.10 $\pm$ 0.52	43.72 $\pm$ 0.58	93.89 $\pm$ 0.42	42.77 $\pm$ 1.65	95.89 $\pm$ 0.26
64	90.59 $\pm$ 0.16	55.15 $\pm$ 0.49		42.24 $\pm$ 0.75	53.40 $\pm$ 1.71		92.95 $\pm$ 0.42	40.14 $\pm$ 0.31	90.32 $\pm$ 0.37	39.94 $\pm$ 0.62	96.01 $\pm$ 0.76
32	72.16 $\pm$ 1.40	48.62 $\pm$ 0.58		41.15 $\pm$ 0.80	43.11 $\pm$ 0.63		91.39 $\pm$ 1.80	37.56 $\pm$ 0.65	74.26 $\pm$ 1.33	37.36 $\pm$ 0.80	96.00 $\pm$ 0.14
16	65.61 $\pm$ 0.51	43.68 $\pm$ 1.20		40.54 $\pm$ 0.31	42.18 $\pm$ 0.40		69.41 $\pm$ 0.98	34.19 $\pm$ 1.47	65.76 $\pm$ 1.65	34.37 $\pm$ 1.70	91.29 $\pm$ 1.93
8	47.64 $\pm$ 1.80	43.79 $\pm$ 1.87		38.42 $\pm$ 1.50	40.96 $\pm$ 0.44		63.37 $\pm$ 1.41	33.08 $\pm$ 0.56	59.33 $\pm$ 0.79	35.96 $\pm$ 1.00	67.81 $\pm$ 0.48
4	38.46 $\pm$ 1.02	44.91 $\pm$ 1.62		36.59 $\pm$ 0.51	38.18 $\pm$ 1.09		55.84 $\pm$ 0.98	32.82 $\pm$ 0.49	50.04 $\pm$ 0.14	33.89 $\pm$ 1.64	46.45 $\pm$ 1.44
2	36.16 $\pm$ 1.43	37.12 $\pm$ 1.89		35.12 $\pm$ 0.51	36.51 $\pm$ 0.75		42.21 $\pm$ 0.16	31.76 $\pm$ 1.03	44.38 $\pm$ 1.82	34.72 $\pm$ 1.94	42.94 $\pm$ 1.22
1	33.82 $\pm$ 0.88	33.80 $\pm$ 1.22		33.90 $\pm$ 1.10	31.94 $\pm$ 1.70		32.38 $\pm$ 0.36	29.28 $\pm$ 1.79	41.83 $\pm$ 0.14	32.71 $\pm$ 1.29	32.43 $\pm$ 0.95

According to the results lowest IC₅₀ values were shown by RMI, RWI, and GMI, 7.378, 16.82 and 17.56 µg/mL respectively while ascorbic acid has

an IC₅₀ value of 6.784 µg/mL. Thus, the extracts RMI, RWI and GMI were selected for further analysis (Table 6).

Table 6: IC₅₀ values of the plant extracts according to the results of DPPH radical scavenging activity

Name of the plant extract	GMI	GMO	GWI	GWO	RMI	RMO	RWI	RWO	AA
IC ₅₀ value (µg/ml)	17.56	67.73	176.1	94.43	7.378	280.3	16.82	335.1	6.784

Sun protection activity

Plant extracts of RMI and GMI exhibited SPF values of 30.97 ± 0.11 and 29.88 ± 0.02 at the concentration of 1024 µg/mL respectively while the other extracts gave SPF values less than 30 while RMO had the lowest SPF value of 6.39 ± 0.68 at the

concentration 1024 µg/mL. The plant extracts which showed SPF values equal or greater than 30 were considered to have higher sun protection activity. Therefore, the extracts RMI and GMI which exhibited highest SPF values at the concentration of 1024 µg/mL were selected for further analysis.

Table 7: SPF values of the plant extracts of *M. zeylanica* bark

Concentration (µg/ml)	GMI	GMO	GWI	GWO	RMI	RMO	RWI	RWO
1024	29.88±0.02	24.24±0.73	23.77±0.02	24.70±0.96	30.97±0.11	6.39±0.68	21.05±0.89	21.10±0.49
512	29.37±0.10	17.07±0.04	22.10±0.86	16.73±0.03	29.81±0.11	3.25±0.61	12.53±0.74	11.13±0.79
256	21.04±0.05	17.58±0.05	21.04±0.05	7.83±0.60	20.22±0.11	1.69±0.58	6.75±0.59	5.07±0.42
128	9.75±0.04	8.77±0.04	9.75±0.04	3.66±0.28	12.70±0.10	0.88±0.56	3.57±0.56	2.46±0.48
64	4.18±0.48	3.20±0.48	4.18±0.48	1.43±0.15	6.22±0.11	0.49±0.53	1.79±0.38	1.35±0.11
32	3.62±0.47	2.64±0.47	3.62±0.47	0.79±0.12	3.41±0.11	0.45±0.57	0.83±0.22	0.67±0.10
16	4.33±0.39	3.35±0.39	4.33±0.39	0.36±0.08	1.34±0.11	0.46±0.55	0.56±0.56	0.24±0.06
8	3.40±0.45	2.42±0.45	3.40±0.45	0.26±0.03	0.55±0.11	0.44±0.57	0.14±0.04	0.12±0.04
4	3.30±0.00	2.30±0.04	3.55±0.43	0.20±0.03	0.51±0.10	0.15±0.03	0.14±0.06	0.08±0.02
2	0.50±0.12	0.53±0.14	0.51±0.14	0.31±0.03	0.58±0.11	0.15±0.06	0.15±0.05	0.07±0.02
1	0.66±0.25	0.58±0.08	0.60±0.14	0.20±0.05	0.67±0.11	0.12±0.02	0.12±0.03	0.11±0.02

Bioactivity guided fractionation

Using column chromatography, bioactivity-guided fractionation was completed for the extracts RMI, GMI, and RWI. Antioxidant and sun protection

activity was assessed in the respective pooled fractions with a comparable TLC profile. Fractions 01 and 02 for all three extracts had R_f values ranging from 0.6 to 0.8 and 0.2 to 0.4, respectively (Figure 1)

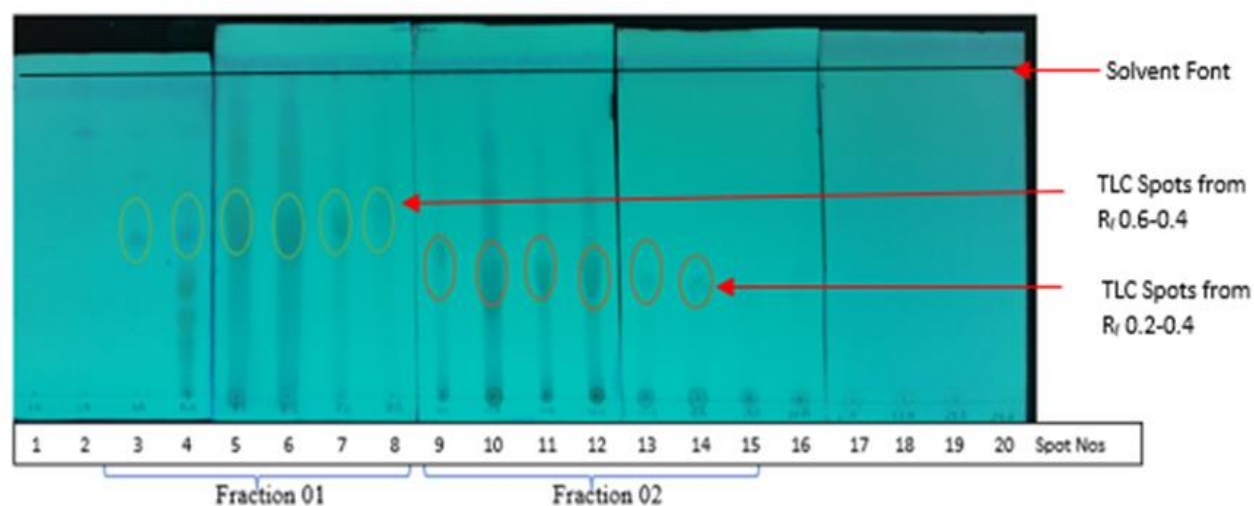


Figure 1: TLC profile of the separated fractions of GMI

Antioxidant activity of the separated fractions

The crude extracts were separated from column chromatography, and 06 fractions were obtained

(Table 8). The antioxidant activity of the fractions were evaluated using DPPH radical scavenging assay.

Table 8: Fractions obtained for plant extracts with potent antioxidant activity

Name of the crude extract	Name of the separated fractions obtained
Rambukkana Methanol Inner Bark (RMI)	RMI Fraction 01
	RMI Fraction 02
Galle Methanol Inner Bark (GMI)	GMI Fraction 01
	GMI Fraction 02
Rambukkana Water Inner Bark (RWI)	RWI Fraction 01
	RWI Fraction 02

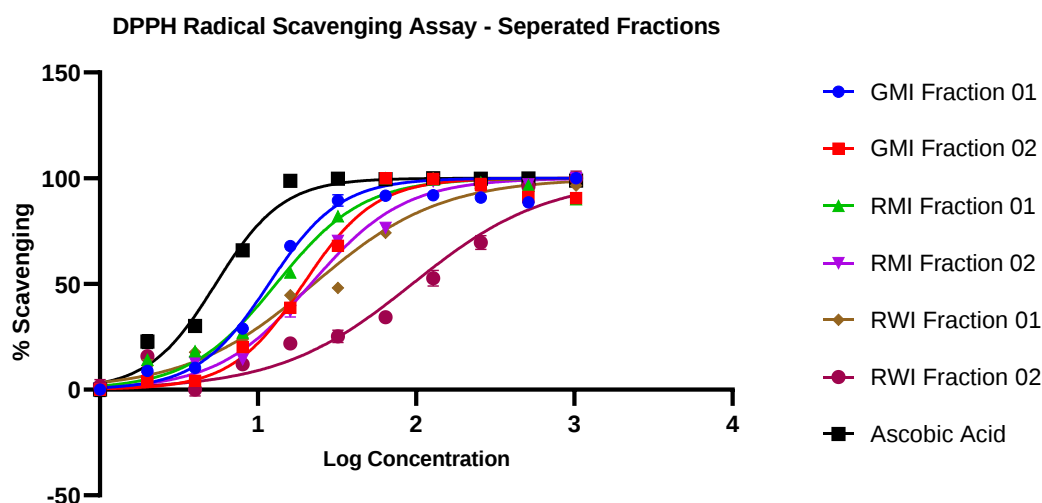
GMI fraction 01 has shown highest percentage scavenging activity (95.19 ± 0.40 %) followed by RWI fraction 01 (87.67 ± 0.26 %) and RMI fraction 01 (86.95 ± 0.89 %) at the concentration of 1024 $\mu\text{g/mL}$. The positive control ascorbic acid has shown a percentage scavenging activity of $96.44 \pm$

0.30 % at the concentration of 1024 $\mu\text{g/mL}$ (Figure 2).

According to the results Ascorbic acid, positive control showed an IC_{50} values of 5.399 $\mu\text{g/ml}$ while fraction 01 of GMI and RMI had IC_{50} values of 11.71 and 13.00 $\mu\text{g/mL}$ respectively (Table 9).

Table 9: IC₅₀ values of the separated fractions according to DPPH radical scavenging activity

Name of the Fraction	GMI Fraction 01	GMI Fraction 02	RMI Fraction 01	RMI Fraction 02	RWI Fraction 01	RWI Fraction 02	Ascorbic Acid
IC ₅₀ Value $\mu\text{g/ml}$	11.71	19.36	13.00	21.58	22.48	94.48	5.399

**Figure 2:** DPPH assay for separated fractions and standard drug

Sun protection activity of the separated fractions

SPF results of the separated fractions of GMI and RMI showed that fraction 01 of each extract have shown SPF values of 30.19 ± 1.35 and 30.63 ± 0.19 (Table 10).

Table 10: Results of the SPF values of the separated fractions of

Concentration ($\mu\text{g/mL}$)	GMI Fraction 01	GMI Fraction 02	RMI Fraction 01	RMI Fraction 02
1024	30.19 ± 1.35	28.66 ± 1.13	30.63 ± 0.19	28.80 ± 1.59
512	23.57 ± 1.04	17.01 ± 0.04	30.38 ± 0.59	14.58 ± 1.00
256	11.74 ± 1.05	13.90 ± 0.05	17.30 ± 0.08	10.96 ± 1.67
128	05.41 ± 1.25	08.77 ± 0.04	11.45 ± 0.53	12.61 ± 0.33
64	02.17 ± 1.20	03.20 ± 0.48	06.16 ± 0.06	02.77 ± 0.51
32	01.24 ± 0.15	02.64 ± 0.47	03.34 ± 0.06	03.19 ± 0.04
16	01.13 ± 0.28	02.59 ± 0.39	01.27 ± 0.06	01.29 ± 0.14
8	00.61 ± 0.03	02.42 ± 0.45	00.50 ± 0.06	01.11 ± 0.29
4	00.65 ± 0.06	01.76 ± 0.04	00.46 ± 0.08	01.09 ± 0.16
2	00.52 ± 0.05	00.70 ± 0.43	00.47 ± 0.02	00.96 ± 0.30
1	00.14 ± 0.14	00.78 ± 0.43	00.55 ± 0.03	00.99 ± 0.20

Discussion

The chemical diversity of the natural products obtained from medicinal plants provides unlimited opportunities for new drug leads. Thus, screening the chemical diversity and seeking therapeutic drugs from natural products have drawn the attention of many researches (Sasidharam et al., 2011). *M. zeylanica* which is endemic to Sri Lanka had been used in the traditional ayurvedic system prevailing in Sri Lanka to treat various diseases. Therefore, the present study investigated the antioxidant and sun protection activity of the bark of this plant.

To examine both phytochemicals and biological activity, plant bark was extracted using three distinct solvents: hexane, methanol, and water. Notably, in this investigation, extracts from polar solvents demonstrated the highest yields, whereas extracts from the non-polar solvent displayed the lowest yield percentages indicating that variance of yield depends on the polarity.

Polyphenols possess antioxidant (Scalbert et al., 2005a), antimicrobial (Daglia, 2012), anti-atherosclerosis and antimutagenic/anticarcinogenic properties (Han et al., 2007). Studies have shown that flavonoids also exhibit biological activities like antioxidant, vasodilating, antiallergenic and antiviral actions (Pietta, 2000). Thus, the presence of polyphenols and flavonoids can suggest the possible biological activities that could be present in the plant bark (Scalbert et al., 2005b).

The current study has shown that the methanolic inner bark extracts from both areas and aqueous inner bark extract collected from Rambukkana area had the highest antioxidant activity potential. A study done by Udem et al, 2018 in Nigeria have revealed that best percentage scavenging activity was obtained for *M. indica* leaf, stem-bark and root-bark ethanol extracts (100 mg/mL) with values 79.09 ± 0.42 %, 59.86 ± 0.32 % and 80.70 ± 0.42 % respectively confirming the results of the present study. Out of the separated fractions, fraction 01 of GMI and RMI had lower IC₅₀ values. Low IC₅₀ value means that the drug is potent at low concentrations, and thus will show lower systemic toxicity when administered to the patient (Berrouet et al., 2020). These findings confirm that the phytochemicals responsible for antioxidant activity are prominent in the separated

fraction 01 of methanolic extracts of inner barks in which the phytochemical composition needed to be determined further.

In conjunction with other bioactivities, the presence of antioxidant activity in the plant extracts can be employed in the development of medications. Since the oxidative stress caused by hyperglycemia can be treated by antioxidants, several studies have demonstrated that plant medicines with antioxidant properties are useful in treating diabetes (Bajaj & Khan, 2012; Rajendrian et al., 2018). Further antioxidants are shown to be effective in antiaging products since antioxidants can neutralize the free radicals and reduce the production of activator protein 01, which is contributing to the wrinkle formation (Palmer & Kitchin, 2010)

The skin cancer foundation has reported that a SPF value of 15 or higher as acceptable UVB protection for normal everyday activity and SPF 30 or higher acceptable for intense outdoor exposure (Arogba & Omede, 2012). The methanolic extracts of the inner barks of *M. zeylanica* showed the highest SPF value while the fraction 1 of methanolic extract also demonstrated SPF values greater than 30. Therefore, these fractions were also considered to have the sun protection activity. It was further supported by the findings of the methanolic extracts of matured plant leaves obtained from *M. indica* had high SPF value of 38.67 at the concentration of 2.0 mg/mL (De Silva et al., 2019). Thus, it is evident that various plant parts of *Mangifera* possess sun protection activity. Therefore, methanolic extract of inner bark of *M. zeylanica* can be used for development of sun protection formulations.

A sunscreen product should be able to prevent sunburn and other skin damage caused by UV radiation. The harmful effects of the UV radiation can occur in the range of 290 nm to 400 nm. Therefore, a sunscreen product needs to have a broader absorbance between 290 nm to 400 nm. SPF value quantitatively measures the effectiveness of potential sunscreen product/plant extracts. This gives a measurement of the amount of solar energy needed to produce an effect on the protected skin. Therefore, higher the SPF value higher the solar energy needed to produce sunburns.

Conclusion

The investigation into the pharmacological activity of *M. zeylanica* bark has shown potential antioxidant and sun protection properties with significant implications for pharmaceutical and cosmetic industries. The findings of the study hold substantial potential for the development of medications, especially for addressing oxidative stress-related conditions like diabetes and anti-aging products. Further research and identification of active compounds within these extracts are warranted to harness their full potential and advance scientific understanding in this field.

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Conflicts of interest

The authors declare no conflicts of interest

References

- Arogba, S., & Omede, A. (2012). Comparative antioxidant activity of processed mango (*Mangifera indica*) and bush mango (*Irvingia gabonensis*) kernels. *Nigerian Food Journal*, 30(2), 17–21.
- Bajaj, S., & Khan, A. (2012). Antioxidants and diabetes. *Indian journal of endocrinology and metabolism*. 16 (Suppl 2), S267.
- Berrouet, C., Dorilas, N., Rejniak, K. A., & Tuncer, N. (2020). Comparison of drug inhibitory effects (IC50) in monolayer and spheroid cultures. *Bulletin of mathematical biology*. 82(6), 68.
- Bhairam, M., Roy, A., Bahadur, S., Banafar, A., & Turkane, D. (2013). Standardization of herbal medicines—an overview. *Journal of Applied Pharmaceutical Research*, 1(1), 14–21.
- Biswas, N. N., Acharzo, A. K., Anamika, S., Khushi, S., & Bokshi, B. (2017). Screening of Natural Bioactive Metabolites and Investigation of Antioxidant, Antimicrobial, Antihyperglycemic, Neuropharmacological, and Cytotoxicity Potentials of *Litsea polyantha* Juss. Ethanollic Root Extract. *Evidence-Based Complementary and Alternative Medicine*.
- Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current opinion in biotechnology*, 23(2), 174–81.
- De Silva, D., Bandara, L., Samanmali, B., Ratnasooriya, W., Pathirana, R., & Abeysekara, W. (2019). Investigation of sun screening and antioxidant activity of *M. indica* ver “Willard.” *Journal of Pharmacognosy and Phytochemistry*. 8(4), 1130–3.
- Ediriweera, M. K., Tennekoon, K., Samarakoon, S., Thabrew, I., & De Silva, E. (2016a). Cytotoxic and apoptotic effects of the bark of two common mango (*Mangifera indica*) varieties from Sri Lanka on breast and ovarian cancer cells. *British Journal of Pharmaceutical Research*, 10(2), 1–7.
- Ediriweera, M. K., Tennekoon, K.H., Samarakoon, S. R., Thabrew, I., & Dilip De Silva, E. (2016b). A study of the potential anticancer activity of *Mangifera zeylanica* bark: Evaluation of cytotoxic and apoptotic effects of the hexane extract and bioassay-guided fractionation to identify phytochemical constituents. *Oncology letters*, 11(2), 1335–44.
- Gul, R., Jan, S.U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. *The Scientific World Journal*.
- Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance. *International journal of molecular sciences*, 8(9), 950–88.
- Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. (2019). Preliminary Analysis of Phytoconstituents and Evaluation of Anthelmintic property of *Cayratia auriculata* (*In vitro*). *Maedica*, 14(4), 350.
- Kaur, C. D., & Saraf, S. (2010). In vitro sun protection factor determination of herbal oils used in cosmetics. *Pharmacognosy research*, 2(1), 22.
- Khare, C. P. (Ed) (2004). Indian herbal remedies: rational Western therapy, ayurvedic, and other traditional usage, Botany. *Springer science & business media*.
- Kumar, A., Jha, K. K., Kumar, D., Agrawal, A., & Gupta, A. (2012). Preliminary phytochemical analysis of leaf and bark (mixture) extract of

- Ficus infectoria* plant. *The Pharma Innovation*, 1(5, Part A), 71.
- Malsawmtluangi, C., Nath, D. K., Jamatia, I., Zarzoliana, E., & Pachuau, L. (2013). Determination of Sun Protection Factor (SPF) number of some aqueous herbal extracts. *Journal of Applied Pharmaceutical Science*, 3(9), 150-151.
- Mamta, S., & Jyoti, S. (2012). Phytochemical screening of *Acorus calamus* and *Lantana camara*. *International Research Journal of Pharmacy*, 3(5), 324-6.
- Mansur, J.de S., Breder, M. N. R., Mansur, M. C. d'Ascensão., & Azulay, R. D. (1986). Determination of the sun protection factor by spectrophotometry. *Anis Brasileiros de Dermatologia*, 121-4.
- Palmer, D. M., & Kitchin, J. S. (2010). Oxidative damage, skin aging, antioxidants and a novel antioxidant rating system. *Journal of Drugs in Dermatology*, 9(1), 11-5.
- Pietta, P. G. (2000). Flavonoids as antioxidants. *Journal of natural products*, 63(7), 1035-42.
- Rajendiran, D., Packirisamy S., Gunasekaran K. (2018). A review on role of antioxidants in diabetes. *Asian Journal of Pharmaceutical and Clinical Research*. 11(2), 48-53.
- Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Latha, L. Y. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of traditional, Complementary and Alternative Medicines*, 8(1).
- Sayre, R. M., Agin, P. P., Le Vee, G. J., & Marlowe, E. (1979). A comparison of in vivo and in vitro testing of sun screening formulas. *Photochemistry and Photobiology*, 29(3), 559-66.
- Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005a). Polyphenols: antioxidants and beyond. *The American Journal of Clinical Nutrition*, 81(1), 215S-217S.
- Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005b). Dietary polyphenols and the prevention of diseases. *Critical reviews in food science and nutrition*, 45(4), 287-306.
- Udem, G. C., Dahiru, D., & Etteh, C.C. (2018). In vitro Antioxidant Activities of Aqueous and Ethanol Extracts of *Mangifera indica* Leaf, Stem-Bark and Root-Bark. *Pharmacognosy Communications*. 8(3).
- Weerarathne, W., Samarajeewa, P., & Nilanthi, R. (2005). Genetic diversity of Etamba in Sri Lanka. *Tropical Agricultural Research and Extension*, 8, 107-12.