



## Antioxidant, $\alpha$ -amylase Inhibitory Activities and Photoprotective Properties of Peels of *Nephelium lappaceum* Linn. (Malwana special)

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### ABSTRACT

This study focused on evaluation of antioxidant,  $\alpha$ -amylase inhibitory activities and photoprotective properties of peels of *Nephelium lappaceum* Linn. (rambutan); Malwana special. Methanolic extract of peels was sequentially partitioned in hexane, dichloromethane (DCM) and aqueous methanol. The methanol extract showed a significantly ( $p < 0.05$ ) higher DPPH radical scavenging activity than that of butylated hydroxytoluene. Among the fractions, the highest total phenolic content (TPC) was found in the aqueous methanol fraction. DCM and aqueous methanol fractions were rich in flavonoids. *In vitro*  $\alpha$ -amylase inhibitory activity of the aqueous methanol fraction was also significantly higher than the standard drug, acarbose. Partially purified aqueous methanol fraction of rambutan peels exhibited UV-B absorption with a moderate solar protection factor. The results revealed that the peels of *Nephelium lappaceum* Linn., Malwana special can be considered as a promising source for the development of natural antioxidant, cosmeceutical sunscreen and antidiabetic agents.

**Keywords:** Antioxidant, Phenolics, Flavonoids,  $\alpha$ -amylase inhibition, Photoprotection.

### INTRODUCTION

Reactive oxygen species (ROS) in biological systems include superoxide ( $O_2^{\bullet-}$ ), hydroxyl ( $HO^{\bullet}$ ), singlet oxygen ( $^1O_2$ ) and peroxy ( $RO_2^{\bullet}$ ) radicals<sup>1</sup>. Reactive oxygen species are generated as byproducts during oxidative metabolism in

the mitochondrial electron-transport system and as intermediates in some enzymatic reactions<sup>1</sup>. Besides, excessive exposure to UV radiation can also generate ROS in the skin<sup>2</sup>. An imbalance between ROS and scavenging systems causes oxidative stress and consequently, leading to various chronic diseases including cancer, diabetes mellitus,



and aging in humans<sup>3</sup>. Recent studies have shown the central role of antioxidants in mediating chronic diseases by inhibiting the formation of free radicals and terminating oxidative chain reactions<sup>4</sup>. However, the use of synthetic antioxidants has been questioned due to their possible carcinogenicity and instability<sup>5</sup>. Therefore, it is important to investigate efficient, safe, cost-effective and biologically active phytochemicals to replace synthetic bioactive compounds. Vitamins, phenolics, and carotenoids are three main groups of remarkable antioxidants in fruits and vegetables<sup>6</sup> that are important for our health as they guard the human body from ROS mediated disorders<sup>7</sup>.

Interestingly, not only the fruits and vegetables but also some of their wastes such as peels, seeds, and pomace are known to enrich with many bioactive compounds<sup>8</sup>. Peels of fruits such as mango, grape, mangosteen, and avocado have been reported to contain higher amounts of bioactive compounds<sup>8,9</sup>. Food wastes including fruit and vegetable wastes that end up in the landfills produce methane; a powerful greenhouse gas which affects the climate changes<sup>10</sup>. As the climate changes are posed serious threat to habitats of organisms, this eventually affect the biodiversity. Therefore, valorization of agro-food wastes by extracting valuable bioactive compounds is a worth while strategy to minimize this problem.

*Nephelium lappaceum* Linn. (rambutan) belongs to family Sapinadaceae<sup>11</sup>; closely related to litchi, longan, mangosteen, and durian<sup>12</sup>. Rambutan is native to Southeast Asia. Three rambutan varieties are mainly cultivated in Sri Lanka. They are Malayan Red, Malayan Yellow, and Malwana special<sup>13</sup>. Among these, Malwana special is the most widely grown high-yielding variety<sup>12</sup> and it is famous in export market due to its excellent quality as a fresh fruit<sup>12</sup>. Unfortunately during the fruit season, a vast amount of waste is generated from its peels which eventually create mosquito breeding grounds<sup>6</sup>. Efforts on value addition to peels of rambutan have led to the identification of powerful antioxidants particularly ellagitannins<sup>14,15</sup>. Ellagitannins have exhibited a greater activity than butylated hydroxytoluene (BHT) and vitamin E<sup>14,16</sup> which are also effective in inhibiting digestive enzymes<sup>17</sup>. Photoprotective efficacy of formulations containing crude extract of rambutan peel has also

been recently reported<sup>18</sup>. Apart from the recent investigation on antioxidant and anti-inflammatory activity of aqueous extract of rambutan peels<sup>18</sup>, no studies have been focused on valorization of peels of rambutan variety, Malwana special.

Therefore, the aim of this research was to investigate the potential utilization of by-products (peels) of *Nephelium lappaceum* Linn., Malwana special, a common rambutan variety in Sri Lanka as a source of phytochemicals rich with antioxidant and  $\alpha$ -amylase inhibitory activities and photoprotective properties.

## MATERIALS AND METHODS

### Plant material

Fresh rambutan fruits of Malwana special variety were collected from a commercial cultivation in Western province, Sri Lanka in June 2019. The variety identification was done by the Fruit Research and Development Institute, Horana, Sri Lanka. The peels were removed, washed with tap water, cut into smaller pieces, soaked, and air-dried for 6 days. Dried peels were ground and packed in sealed polythene bags and then kept at 4°C until use.

### Sample preparation

The chemical constituents in powdered rambutan peels (10.0 g) were extracted using a laboratory-scale soxhlet apparatus with 99% methanol (120.0 mL) for 6 h at 60°C until the solvent in the siphon tube become colorless. Then extract was filtered and solvent was removed using a rotary evaporator (IKA RV 10 digital, Germany) at 35°C and freeze-dried using a freeze dryer (LABCONCO, USA) and kept at 4°C until further use.

### Fractionation of methanol extract

Dried methanol crude extract was dissolved in methanol (50 mL) and transferred into 500 mL separatory funnel. Methanol extract was successively partitioned into hexane (40 mL). Resulted methanol layer was mixed with dichloromethane (DCM) (75 × 2 mL). Then 5 mL of distilled water was added to separate the DCM layer from aqueous methanol layer. Resulted fractions (hexane, DCM, aqueous methanol) were separately concentrated and freeze-dried and kept at 4°C until further use.

### DPPH radical scavenging assay

The  $\alpha, \alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) free radical scavenging assay was carried out by following the method reported by Chatatikun and Chiabchalard (2013)<sup>19</sup> with slight modifications. 160  $\mu$ L of various concentrations of the methanol extract, and its fractions (hexane, DCM, and aqueous methanol) and standard (BHT) (250, 125, 62.5, 31.25, 15.63, 7.82, 3.91, 1.95  $\mu$ g/mL) were added to methanolic DPPH (40  $\mu$ L, 0.26 mg/mL). The absorbance was recorded at 517 nm using microplate spectrophotometer (Multiskan go 1.00.40, Thermo scientific) after incubating the well-mixed samples in the dark for 15 minutes. The control consisted of 160  $\mu$ L of methanol and 40  $\mu$ L of DPPH. The percentage inhibition (%) was calculated by using the equation 1.

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

Where,  $A_c$  and  $A_s$  are the absorbance of the control and absorbance of the sample (extracts or standard) respectively. The sample concentration which generates 50% DPPH inhibition ( $IC_{50}$ ) was determined by the plot of percent inhibition versus concentrations of the samples.

### Total phenolic content (TPC)

The total polyphenolic content (TPC) in methanol extract of rambutan peels and its fractions were determined according to the Folin-Ciocalteu (F-C) method<sup>20</sup> with slight modifications. Briefly, 0.10 mL of sample solutions, (1000  $\mu$ g/mL) ( $n = 3$  each) were transferred to 7.9 mL of deionized water, and incubated with 0.5 mL of F-C reagent and 1.5 mL of 200 g/L  $Na_2CO_3$  solution for 2 hours. Then, absorbance was recorded using microplate spectrophotometer at 760 nm. TPC of each sample was calculated using the constructed Gallic acid standard curve ( $R^2 = 0.97$ ).

### Total flavonoid content (TFC)

The total flavonoid content (TFC) in the methanol extract and its fractions were determined by  $AlCl_3$  colorimetric method with slight modifications<sup>21</sup>. Briefly, samples (0.5 mL, 1000  $\mu$ g/mL) ( $n = 3$  each) were incubated with deionized water (2.0 mL) and 5%  $NaNO_2$  (150  $\mu$ L) for 5 min in the dark. Then 10%  $AlCl_3$  (150  $\mu$ L) was added and incubated for 6 minutes. Next, the absorbance of

samples was recorded at 510 nm using microplate spectrophotometer after adding 1 M NaOH (1.0 mL) and distilled water (1.0 mL) to the samples. The blank was prepared with all the reagents except peel extract. The same procedure was carried out for the standard concentration series of quercetin (1000, 800, 500, 400, 250, 200, 125  $\mu$ g/mL) instead of the sample. TFC of each sample was determined by the constructed quercetin standard curve ( $R^2 = 0.98$ ).

### Determination of photoprotective properties Chromatographic fractionation of aqueous methanol fraction

As the aqueous methanol fraction had the highest phenolic content and the highest DPPH free radical scavenging activity, it was selected to evaluate its photoprotective properties. Firstly, aqueous methanol fraction was purified using silica gel column (130 $\times$ 16 mm) by eluting the column with ethyl acetate (100%, 150 mL), ethyl acetate: acetone (1:1, 150 mL), acetone (100%, 200 mL) and ethanol (100%, 100 mL). According to similarities in thin layer chromatography (TLC) profiles of collected fractions, fractions were combined into 3 sub-fractions: Fraction-A (1-60 fractions), Fraction-B (61-80 fractions), and Fraction-C (81-132) and solvents were evaporated under reduced pressure and stored at 4°C until further use. Further, UV-spectra of three sub-fractions were recorded.

### Determination of *In vitro* sun protection factor (SPF)

The estimated sun protection factor (SPF) of fraction-A was determined by a spectrophotometric method<sup>22</sup>, using the equation 2. A commercial sunscreen product containing Avobenzene 3.0%, Homosalate 10.0%, Octisalate 5.0 %, and Octocrylene 10.0% as active ingredients was selected as the references for this study. Fraction-A and the reference sunscreen were dissolved in methanol (99.8% from Sigma-Aldrich) to prepare solutions of 2.0 g/mL. A dilution series of the Fraction-A and reference sunscreen were prepared (0.03, 0.06, 0.13, 0.25, 0.5, 1 mg/mL). UV absorbance of each dilution was determined ( $n=3$ ) from 290 to 320 nm (UV-B region in the spectrum), at 5 nm intervals using the microplate spectrophotometer. Methanol was used as the blank.

$$SPF = CF \times \sum_{290}^{220} EE(\lambda) \times I(\lambda) \times abs(\lambda) \quad (2)$$

Where, CF = Correction factor (equal to 10). It was estimated from the standard formulation containing 8% homosalate with SPF of 4<sup>18</sup>. EE ( $\lambda$ ) is the erythemal effect of radiation at the wavelength; I ( $\lambda$ ) is the intensity of light at the wavelength I; abs ( $\lambda$ ) is the absorbance reading of plant extract or reference sunscreen at the wavelength  $\lambda$ . The values of EE  $\times$  I were predetermined<sup>23</sup>.

**In vitro  $\alpha$ -amylase inhibitory activity**

The  $\alpha$ -amylase inhibitory activity of aqueous methanol fraction of rambutan peels was evaluated by the method reported<sup>17</sup> with few modifications. Briefly, the aqueous methanol fraction (80  $\mu$ L, 7.81-1000  $\mu$ g/mL) of peel of rambutan, starch (40  $\mu$ L, 1%, w/v), and amylase in phosphate buffer (40  $\mu$ L) were incubated at 37°C for 10 minute. Next, dinitrosalicylic (DNS) reagent (80  $\mu$ L) was added and incubated at 95°C for another 10 minute. Reaction mixtures were cooled and the absorbances were measured at 540 nm. Acarbose (125-1000  $\mu$ g/mL) was used as the positive control. Sample blanks were prepared by replacing the enzyme with the buffer. For negative control, deionized water was used instead of plant extract. For blank solution of the negative control, distilled water and buffer was used instead of plant extract and enzyme. The  $\alpha$ -amylase inhibitory activity was given as percentage inhibition and it was calculated using the equation 3.

$$\text{Inhibition(\%)} = \frac{(A_{\text{neg.control}} - A_{\text{blank}}) - (A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{neg.control}} - A_{\text{blank}})} \times 100 \quad (3)$$

Where,  $A_{\text{neg.control}}$  is the absorbance of the negative control.  $A_{\text{blank}}$  is the absorbance of the blank.  $A_{\text{sample}}$  is the absorbance of samples containing plant extract/positive control.  $A_{\text{sample blank}}$  is the absorbance of sample blank. The half maximal inhibitory concentrations ( $IC_{50}$ ) were obtained by plotting %  $\alpha$ -amylase inhibition against concentration of extract/standard.

**Table 1:  $IC_{50}$  values of the methanol extract of rambutan peels and its fractions**

	$IC_{50}$ values ( $\mu$ g/mL )			
BHT	methanol extract	Hexane fraction	DCM fraction	Aqueous methanol fraction
13.92 $\pm$ 1.19 <sup>c</sup>	9.70 $\pm$ 0.50 <sup>d</sup>	69.10 $\pm$ 1.08 <sup>a</sup>	20.81 $\pm$ 0.21 <sup>b</sup>	12.04 $\pm$ 0.80 <sup>c</sup>

Values are mean  $\pm$  standard deviation. Different letters (a–d) within the row differs significantly ( $p < 0.05$ ).

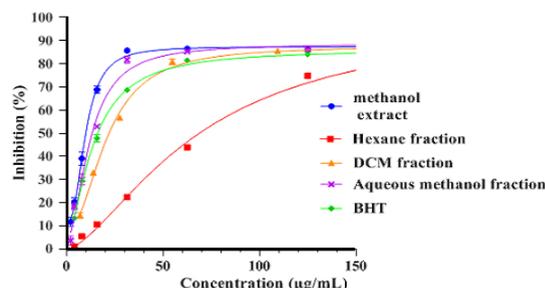
**Statistical analysis**

All the experiments were conducted in triplicate. The results were processed statistically using GraphPad Prism 7.00 Statistic software. All data were reported as mean  $\pm$  SD. Significant differences were assessed from Tukey’s pairwise test after conducting ANOVA5. Values were considered significant at  $p < 0.05$ .  $IC_{50}$  values were obtained from GraphPad prism software by non-linear regression analysis program. Pearson’s correlation analysis was performed among  $1/IC_{50}$  and TPC or TFC.

**RESULTS AND DISCUSSION**

**In vitro antioxidant activity: DPPH free radical scavenging activity**

Most natural antioxidants are known to have similar or higher antioxidant capacities than synthetic antioxidants (e.g. BHT, 2-3-ter-butyl-4-methoxyphenol (BHA), and ter-butylhydroquinone (TBHQ))<sup>24</sup>. In the present study, DPPH radical scavenging assay was used to evaluate the antioxidant activity of the methanol extract of rambutan peels and its fractions. DPPH free radical has a deep violet color due to the delocalization of spare electron ( $\lambda_{\text{max}} = 517 \text{ nm}$ )<sup>25</sup>. In the presence of an electron or hydrogen radical donor, DPPH converts into its diamagnetic reduced form leading to a color change from violet to yellow<sup>26</sup>. Fig. 1 illustrates the percentage inhibition of DPPH vs. concentration of methanol extract and its fractions alongside positive control, BHT.  $IC_{50}$  values of each fractions are given in Table 2.



**Fig. 1. DPPH radical scavenging activity (inhibition %) of methanol extract and its hexane, DCM and aqueous methanol fractions. BHT was used as the standard antioxidant (n=3)**

According to the Fig. 1, methanol extract and all of its fractions exhibited DPPH radical scavenging activity. Among the fractions, aqueous methanol fraction exhibited the highest radical scavenging activity with the highest percent inhibition ranging from 18.4 to 87.5% for concentrations from 3.9 to 150 µg/mL. The positive control, BHT, showed values of 13.2-84.3% for the same concentration range. Further, methanol extract, DCM fraction, and aqueous methanol fraction reached to >80% of DPPH radical scavenging activity at the concentration range 50-100 µg/mL.

The IC<sub>50</sub> of methanol extract exhibited a significantly ( $p < 0.05$ ) higher radical scavenging activity than BHT. There was a significant difference ( $p < 0.05$ ) between IC<sub>50</sub> values of methanol extract and its fractions (Table 1). Among the fractions, aqueous methanol fraction had the lowest IC<sub>50</sub> followed by the DCM and hexane fractions respectively.

#### Total phenolic content (TPC) and total flavonoid content (TFC)

High antioxidant activity of many fruits and their wastes are associated with phenolic compounds. Flavonoids are reported as one of the most important natural phenolic compounds with various biological and chemical activities<sup>27</sup>. TPC and TFC of methanol extract of rambutan peels and its fractions with their corresponding yields are presented in Table 2.

**Table 2: Mean values of yield, total polyphenolic content, and total flavonoid content of methanol extract of rambutan peels and its fractions**

Extract/fraction	Yield (g)	TPC*	TFC*
Methanol extract	3.45	318.59 ± 0.09 <sup>a</sup>	245.39 ± 4.83 <sup>a</sup>
Hexane fraction	0.04	38.80 ± 0.16 <sup>b</sup>	40.98 ± 4.17 <sup>b</sup>
DCM fraction	0.13	103.89 ± 0.51 <sup>c</sup>	136.41 ± 32.25 <sup>c</sup>
Aqueous methanol fraction	2.38	141.73 ± 18.66 <sup>d</sup>	110.67 ± 1.43 <sup>c</sup>

Total flavonoid content (TFC) is expressed in mg quercetin equivalents/g of dry weight of extract. Values are given as mean ± standard deviation. Different letters (a–d) within the same column indicate significant difference at  $p < 0.05$ .

Total phenolic content of methanol extract and its fractions obtained from linear regression equation of Gallic acid calibration curve ( $y = 0.989x + 0.057$ ,  $R^2 = 0.97$ ) and expressed in mg of gallic acid equivalents (GAE)/g of dry weight of extract.

When considering the TPC of fractionated methanol extract (Table 2), there was a significant difference ( $p < 0.05$ ) of phenolic content in 3 fractions. Phenolics have been retained preferably in the aqueous methanol layer ( $141.73 \pm 18.66$  mg GAE/g of dry weight of extract) due to the polar nature of the phenolics. A considerable amount of phenolics have also been fractionated into DCM ( $103.89 \pm 0.51$  mg GAE/g of dry weight of extract) from methanol extract. Studies on other varieties of rambutan have identified peel as a good source of natural antioxidants with high phenolic content<sup>15,14,20</sup>. It is reported that TPC of peel of rambutan from Rongrien and Seechompoo cultivars increases during fruit development and the highest content ( $402$  mg GAE/g of dry extract) obtains at the harvest stage<sup>20</sup>. Phenolic profiles of a particular variety of plants can be altered by geographical conditions, differences in cultivars, properties of soil, methods of extraction, and analysis<sup>6,28</sup>. Studies on peels of different mango genotypes in Sri Lanka have also reported the influence of genotype on phenolic content<sup>29</sup>.

The TFC of methanol extract and its fractions obtained from linear regression equation of quercetin calibration curve ( $y = 0.0009x + 0.110$ ,  $R^2 = 0.98$ ) and expressed in quercetin equivalents (QE) (Table 2). TFC in methanol extract was  $245.39 \pm 4.83$  mg QE/g of dry extract. TFC of hexane, DCM, and aqueous methanol fractions were  $40.98 \pm 4.17$  mg QE/g of dry weight of extract,  $136.41 \pm 32.25$  mg QE/g of dry weight of extract, and  $110.67 \pm 1.43$  mg QE/g of dry weight of extract respectively. There was no significant difference ( $p > 0.05$ ) in TFC of DCM and aqueous methanol fraction; indicating that the peel of Malwana rambutan may contain moderately polar and highly polar flavonoids that retain in the DCM and aqueous methanol layer respectively. In line with our findings, a previous study has also reported that flavonoid content of aqueous extract of peel of Malwana rambutan was  $375.0 \pm 13.2$  mg QE/g<sup>29</sup> of dried plant material. Therefore, the results confirmed that the peel of Malwana rambutan variety is enriched with flavonoids.

#### Correlation between TPC, TFC, and antioxidant activities

Strong positive correlations were observed between DPPH radical scavenging activity and the

TPC and TFC with Pearson's correlation coefficients ( $r$ ) of 0.91 and 0.85 respectively (Table 4), suggesting that phenolic phytochemicals including flavonoids have contributed to strong antioxidant activity of the methanol extract of rambutan peels. Ellagitannin are the most active antioxidant identified from methanol extract obtain from cold extraction of peels of rambutan that grown in Thailand<sup>13</sup>. According to our knowledge, this is the first correlation study of TPC, TFC, and antioxidant activities of peels of Sri Lankan variety of rambutan; Malwana special.

**Table 3: Statistical interpretation of the correlation of the TPC and TFC with the DPPH radical scavenging activity ( $1/IC_{50}$ )**

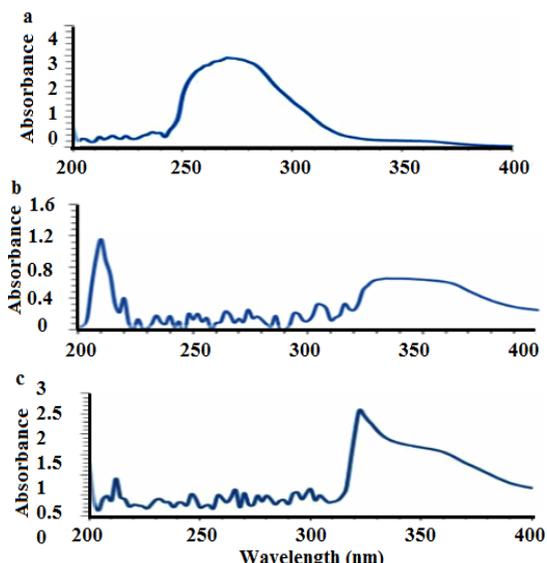
Parameter*	$1/IC_{50}$ vs. TPC	$1/IC_{50}$ vs. TFC
Pearson correlation coefficient ( $r$ )	0.91	
	0.84	
$R^2$	0.83	0.71
$p$ (two tailed)	<0.01	<0.01
Number of xy pairs	10	10

$p < 0.01$  indicates that correlation at the 0.01 level (two tailed) is significant ( $n=10$ ).  $r$ : Pearson's correlation coefficient.  $R^2$ : regression values

### Photoprotective Properties

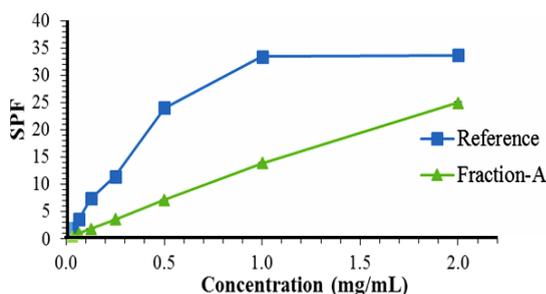
The wavelength region of UV-radiation is 100 nm-400 nm and which can be classified into 3 types, UV-A ( $\lambda=320-400$  nm), UV-B ( $\lambda=290-320$  nm), and UV-C ( $\lambda=100-290$  nm). UV-B radiation may induce skin aging and melanoma and non-melanoma skin cancer<sup>30</sup>. Also, UV-B radiation is considered as one of the main causes of skin damage leading to sunburn<sup>31</sup>. Recent studies suggest that phytochemicals in fruits such as green tea polyphenols and grape seed proanthocyanidins are efficient photoprotective agents<sup>23</sup>. Since aqueous methanol fraction has enriched with phenolics and flavonoids, we determined its photoprotective properties.

Firstly, the aqueous methanol fraction of the rambutan peel extract was purified into 3 sub-fractions (Fraction A, B, C) using silica gel column chromatography. According to UV-spectra, Fraction-A, showed a broad absorbance peak (Fig. 2(a)) near UV-B (280-315 nm) indicating the presence of chemical compounds with UV-B photoprotective properties.



**Fig. 2. UV-spectra (200-400 nm) of partially purified aqueous methanol fraction, (a) fraction-A, (b) fraction-B, (c) fraction-C**

Then, the efficacy of UV-B protection of fraction-A was measured using an *In vitro* method developed by Mansur *et al.*, (1986) as it showed a significant correlation with data obtained *In vivo*<sup>21</sup>. The photoprotective properties of fraction-A and the reference were evaluated by determining sun protection factor (SPF) at different concentrations. The SPF can be defined as measure of how well a sunscreen will protect skin from UV-B rays<sup>22</sup>. According to results illustrated in Fig. 3, both the fraction-A and reference exhibited photoprotective properties in dose dependent manner in the concentration range of 0.03-2 mg/mL. Studies have reported that efficacy of UV-filters in sunscreen depends on concentration of photoprotective agents<sup>21</sup>. According to the SPF ratings, 2 at the highest concentration tested (2.0 mg/mL), the reference and fraction-A possess high (SPF =  $33.67 \pm 0.04$ ) and moderate (SPF =  $25.00 \pm 0.11$ ) photoprotective properties respectively.



**Fig. 3. Sun protection factor (SPF) of fraction-A and the reference sunscreen product vs. concentration (mg/mL)**

Further, it has also been noted that the  $IC_{50}$  (DPPH free radical scavenging activity) of fraction-A was  $35.30 \pm 1.32 \mu\text{g/mL}$ , indicating that free radical scavenging activity of fraction-A adds a beneficial property to protect the skin from UV-induced oxidative stress and DNA damage<sup>2</sup>. Therefore, the results revealed that the yellowish fraction (fraction-A) isolated from aqueous methanol fraction has a potential to use in sunscreen products since adding antioxidants to UV-filters in sunscreen has been identified as a novel photoprotective strategy<sup>32</sup>. According to our knowledge, this is the first study on *In vitro* photoprotective ability of peel of *N. lappaceum*, Malwana special along with its antioxidant activity. Therefore, the *In vivo* studies should be carried out to determine the efficacy and safety of these polyphenols to be applied as an active ingredient in cosmetic products.

#### $\alpha$ -amylase inhibitory activity

Diabetes mellitus is one of the prevailing metabolic disorders characterized by high levels of blood glucose.  $\alpha$ -amylase is responsible for hydrolyzing dietary starch mainly to maltose, prior to absorption as glucose. Inhibition of  $\alpha$ -amylase activity result in slow down the hydrolysis of  $\alpha$ -1,4-glycosidic bonds in starch and other oligosaccharides into simple sugars<sup>33</sup> which delays the glucose absorption. Thus, inhibition of  $\alpha$ -amylase activity plays a vital role in the management of diabetes<sup>34</sup>. As natural products rich in polyphenols have beneficial effects in patients with diabetes, in this study the anti-hyperglycemic effects of Sri Lankan variety of rambutan peel apart from its other properties was evaluated.

The aqueous methanol fraction which had the highest TPC was assessed for *In vitro*  $\alpha$ -amylase inhibitory ability by following a method developed by Palanisamy *et al.*, (2010)<sup>19</sup>. Phenolic rich aqueous methanol fraction inhibited  $\alpha$ -amylase ( $IC_{50} = 75.17 \pm 3.40 \mu\text{g/mL}$ ) more effectively than the positive control Acarbose ( $IC_{50} = 171.50 \pm 8.50 \mu\text{g/mL}$ ) indicating that the extract contained bioactive compounds with potential  $\alpha$ -amylase inhibitory activity. There is a synergistic act of different polyphenol compounds on different steps in starch digestion<sup>35</sup>. The high activity of the extract may be due to combination of several phenolic compounds. Previous studies on other varieties of rambutan have also reported the efficacy of rambutan peel extracts compared to acarbose<sup>16</sup>.

#### CONCLUSION

The sequential fractionation of soxlet methanolic extract of peels of *N. lappaceum*, Malwana special into hexane, DCM and aqueous methanol revealed that phytochemicals with antioxidant activity have mainly concentrated into aqueous methanol fraction and showed the highest DPPH radical scavenging activity ( $IC_{50} = 12.04 \pm 0.80 \mu\text{g/mL}$ ) and which was found to be greater than that of BHT ( $IC_{50} = 13.92 \pm 1.19 \mu\text{g/mL}$ ). The results suggested that there is a clear potential for the utilization of peels *N. lappaceum*, Malwana special as a food additive enriched with antioxidants. It was also noted that the peels are rich in phenolic compounds including flavonoids. Among the fractions, the highest TPC of  $141.73 \pm 18.66 \text{ mg gallic acid equivalent/g}$  of dry extract was found to be in aqueous methanol fraction and the DCM and aqueous methanol fractions were rich in flavonoids with TFC of  $136.41 \pm 32.25$ , and  $110.67 \pm 1.43 \text{ mg quercetin equivalent/g}$  of dry extract respectively. Correlation studies revealed that both polyphenols and flavonoids serve as potential antioxidants in methanolic extract of peels of rambutan, Malwana special. Interestingly, it has also been found out that the *In vitro*  $\alpha$ -amylase inhibitory activity of the aqueous methanol fraction of the peels ( $IC_{50} = 75.17 \pm 3.40 \mu\text{g/mL}$ ) was significantly higher than the standard drug acarbose ( $IC_{50} = 171.5 \pm 8.50 \mu\text{g/mL}$ ) suggesting its antidiabetic potential. Further, the partially purified aqueous methanol fraction by column chromatography possess moderate photo protective activity with SPF of  $25.00 \pm 0.11$  compared to reference sunscreen (SPF =  $33.67 \pm 0.04$ ) at the sample concentration of  $2.0 \text{ mg/mL}$ . The synergistic act of UV-B protection with radical scavenging activity of phytochemicals present in peel of rambutan can be considered as a promising natural additive for enhancing photoprotective properties in sunscreen formulations. These results suggest that further studies should be carried out to isolate and characterize phytochemicals in peels of *Nephelium lappaceum* Linn. with antioxidant and  $\alpha$ -amylase inhibitory activities and photoprotective properties which can effectively use in cosmetic and pharmaceutical products.

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**Conflict of interest**

The authors have no conflict of interest to declare.

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