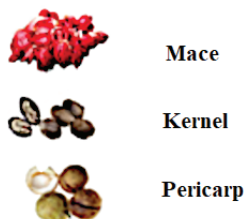


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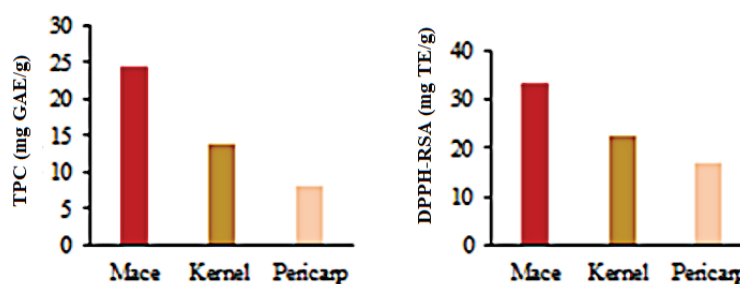
Antioxidant Properties and Proximate Composition of Sri Lankan Nutmeg (*Myristica fragrans* Houtt.)

J. A. M. S. R. Perera, S.P. Rebeira*, H.A.T.P. W. Hettiarachchi, B.D.R. Prasantha and D.V. Jayatilake

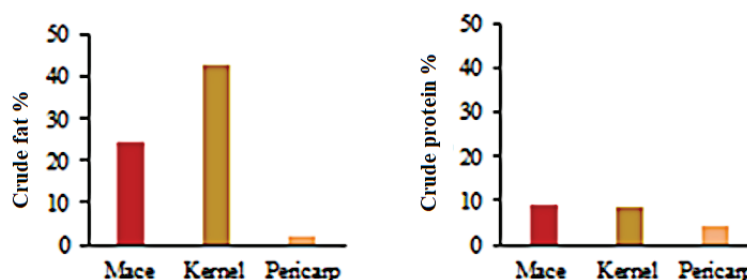
Parts of Nutmeg with Potential Economic Value



Total Polyphenolic Content and DPPH-Radical Scavenging Activity



Crude Fat and Crude Protein Content



Highlights

- Proximate composition and antioxidant potential of Sri Lankan nutmeg were assessed
- Fat content in kernel was significantly higher than mace and pericarp
- Phenolic and DPPH radical scavenging activity of mace was higher compared to kernel and pericarp
- Ferric ion reducing ability (FRAP) values were not significantly different among mace, kernel, and pericarp
- Pericarp has potential for bioactive use in nutraceuticals or preservatives

RESEARCH ARTICLE

Antioxidant Properties and Proximate Composition of Sri Lankan Nutmeg (*Myristica fragrans* Houtt.)

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Abstract: Nutmeg is a high-value spice whose biochemical and nutraceutical properties vary with geographical origin and germplasm. Although the antioxidant properties of Sri Lankan nutmeg have been studied previously, the findings are not representative of the broader germplasm, and the proximate composition of Sri Lankan nutmeg remains unknown. Hence, in the present study, the proximate composition, antioxidant content, and antioxidant activity of the mace, kernel, and pericarp of Sri Lankan nutmeg were assessed using samples collected from Kandy, Matale, and Kegalle districts. Proximate analysis indicated a significant ($p < 0.05$) variation in moisture, crude fat, crude fiber, and ash content among the mace, kernel, and pericarp. Interestingly, the crude fat content of the kernel was two-fold and 21-fold higher compared to the mace and pericarp, respectively. Furthermore, the crude protein content in the mace and kernel was significantly ($p < 0.05$) higher than in the pericarp, showing nearly a two-fold difference. The antioxidant assays revealed that the mace had the highest total phenolic content (TPC: 24.50 mg GAE/g), total flavonoid content (TFC: 93.20 mg RE/g), and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH-RSA: 33.09 mg TE/g). The ferric ion reducing ability, assessed by the FRAP assay, showed no significant ($p > 0.05$) differences among the mace, kernel, and pericarp. Overall, the mace was superior in terms of antioxidant properties, followed by the kernel. Although the pericarp did not contain as high TPC, TFC, and DPPH-RSA levels as the mace and kernel, its electron-donating ability in the FRAP assay indicates the potential value of these otherwise discarded nutmeg by-products. This study highlights the potential applications of nutmeg in industries beyond its traditional use as a spice.

Keywords: Mace; Nutmeg; Pericarp; Proximate composition; Radical scavenging activity

INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt.) is a tropical evergreen tree belonging to the family Myristicaceae. Nutmeg is referred to as *twin spice*, and it carries a distinctive pungent fragrance and a warm, slightly sweet taste. The mace and kernel of nutmeg are commonly used as a spice in many cuisines around the world (Ashokkumar et al., 2022), traditional ayurvedic medicine, and in the pharmaceutical and cosmetics industry (Gupta et al., 2013; Naher et al.,

2014; Okiki et al., 2023). The nutmeg tree is native to the Moluccas in East Indonesia (Van Gils & Cox, 1994; Olaleye et al., 2006; Pal et al., 2011; Ashokkumar et al., 2022; Sundar et al., 2024) and is believed to be introduced to Sri Lanka in the 19th century (Sasikumar, 2021). In Sri Lanka, nutmeg is a common tree crop in the home-gardens of Kandy, Matale, and Kegalle administrative districts (Sarath-Kumara et al., 1985). Although nutmeg is typically cultivated with minimal agronomic inputs, the well-dried, high-quality mace and kernel fetch premium prices in the market. In 2022, nutmeg was cultivated in an extent of 2,936 ha in Sri Lanka (AgStat, 2023), which led to a production of 2,878 t of nutmeg. In 2022, the total export volume in terms of mace and kernel was 2,347 t and this generated an export revenue of LKR 4,315 million (AgStat, 2023). In addition to that, 63 t was exported as nutmeg oil, increasing the total revenue of nutmeg exports to LKR 7,214 million. Nutmeg pericarp is often discarded as waste due to lack of awareness about its industrial applications, however it holds potential as a biomaterial for value addition (Hewage & Vithana, 2013; Suwarda et al., 2021; Shreyas et al., 2023).

Nutmeg is a potential source of antioxidants, where numerous studies have identified bioactive compounds in its mace, kernel, and pericarp (Sulaiman & Ooi, 2012; Gupta et al., 2013; Tan et al., 2013; Assa et al., 2014; and Fernando & Seneviratne, 2019). Nutmeg demonstrates antioxidant activity largely due to the presence of compounds like β -caryophyllene and eugenol, which possess hydrogen atoms at allylic or benzylic positions, and these functional groups enable the efficient removal of hydrogen atoms, enhancing the compounds' antioxidant potential (Naeem et al., 2016). Gupta et al. (2013) evaluated the antioxidant activity of the nutmeg seed extracts using various solvents, including acetone, ethanol, methanol, butanol, and water, where in all extracts significant antioxidant activity was exhibited, confirming that nutmeg extract has potential natural antioxidant activity. In addition to antioxidants, moisture, carbohydrates, proteins, crude fiber, crude ash, and mineral content of nutmeg seeds have been assessed

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in several studies (Okonkwo & Ogu, 2014; Thomas & Krishnakumari, 2015; Asika et al., 2016; Adegbite et al., 2021; and Okiki et al., 2023), and some of these parameters were also assessed in the nutmeg pericarp by Simenthy (2015). These proximate components are crucial for the food industry, serving various purposes, including product development, quality control, and meeting regulatory requirements (Parimelazhagan, 2016). Therefore, knowing the proximate composition of nutmeg, especially that of the pericarp, which is generally discarded in large quantities as waste, is important for the optimal utilization of the nutmeg harvest.

Climatic and soil factors, agronomic practices, plant physiology, and the genetic background may influence the biochemical profiles of nutmeg. Hence, the profiles cannot be generalized for nutmeg, as it has vastly different genetic backgrounds and is grown in variable environments. Most of the studies on antioxidant properties and proximate analysis is done based on the nutmeg germplasm of India, Indonesia, Malaysia, and Nigeria. In Sri Lanka, limited studies have been conducted to evaluate the available antioxidant properties and proximate composition of the local nutmeg germplasm. Fernando & Seneviratne (2019) assessed the antioxidant properties of kernel, mace, and peel samples of Sri Lankan nutmeg. However, this study involved samples from a single location in Matale district, and adopted different drying methods from machine drying (kernel and mace) to sun-drying (pericarp) for different parts, making comparison of antioxidant properties difficult between the components. Furthermore, drying methods may significantly affect the total antioxidant properties of nutmeg due to the temperature susceptibility of the various components of antioxidants in the plant material (Réblová, 2012). Hence, the used method may have an impact on the final assessment. Therefore, there is a need to assess the proximate components and antioxidant properties of Sri Lankan nutmeg to understand its potential. The present study provides a comprehensive analysis of the antioxidant properties and proximate composition of Sri Lankan nutmeg, focusing on air-dried mace, kernel, and pericarp from freshly harvested mature fruits collected from Kandy, Matale, and Kegalle districts, where nutmeg is extensively cultivated.

MATERIALS AND METHODS

Sample preparation

Split-mature nutmeg samples were collected from home gardens of extensively cultivated districts in Sri Lanka: Kandy, Matale, and Kegalle. To ensure representative sampling, five composite samples were prepared, with each composite consisting of 10–15 fruits collected from at least three different trees. Fruits were surface sterilized with 70% ethanol and were separated into mace, kernel, and pericarp. The samples were air-dried in the laboratory, keeping away from direct sunlight at an ambient temperature ($28 \pm 2^\circ\text{C}$; RH: $85 \pm 3\%$) for 14 days. The air-dried mace, kernel, and pericarp samples were ground to a fine powder and stored at 4°C until further analysis. The study focused on three components of nutmeg: mace, kernel, and peel. The five

composite samples were treated as biological replicates for each component. Each biological replicate was further analyzed in triplicate. The mean values obtained from the five biological replicates were used in presenting the results for each component.

Proximate composition of the mace, kernel, and pericarp of nutmeg

Moisture, fat, fiber, ash, protein, and carbohydrate content of mace, kernel, and pericarp samples were assessed in triplicate (Horwitz & Latimer, 2010). The moisture content was determined on a dry basis gravimetrically by drying the samples in an oven (SPF 600, Sibata, Japan) at 105°C . The ash content of the samples was assessed by incinerating the samples in a muffle furnace (Hobersal HD-150, Spain) at 550°C . Crude fats were determined using moisture-free samples extracted with petroleum ether using a Soxhlet extraction apparatus at $60\text{--}80^\circ\text{C}$. The extracted fat was separated using a rotary evaporator (Buchi R-114, Japan). The crude fat content (dry basis) was calculated as a percentage with respect to the initial moisture-free sample. To determine crude fiber, moisture-free and ether-extracted samples were subjected to digestion using a diluted sulphuric acid and a diluted base, sodium hydroxide. The undigested residue obtained after digestion was then ignited, and the weight loss following ignition was recorded. Crude fiber content (dry basis) was expressed as a percentage, with respect to the initial moisture-free and fat-free sample weight. Further, the crude protein content was determined using the micro-Kjeldahl extraction method (Horwitz & Latimer, 2010). The carbohydrate content was determined by difference, calculated as the remaining percentage after subtracting the contributions of moisture, fat, fiber, ash, and protein from 100%. This approach assumes that the sum of moisture, fat, fiber, and protein components accounts for all measurable constituents, and the remaining portion represents the carbohydrate fraction. Results are reported as a percentage of the total sample weight.

Antioxidant properties of the mace, kernel, and pericarp of nutmeg

Sample extracts were prepared based on the methods described by Tan et al. (2013) with slight modifications. Air-dried samples weighing 0.5 g were mixed with 12.5 mL of 80% methanol in a conical flask. The content was stirred manually at a 12-hour interval for 72 hours. The extract was centrifuged at 4,000 rpm for 15 min, and the supernatant was extracted and stored at 4°C until further analysis. The extract was used to determine the total phenolic content (TPC), total flavonoid content (TFC), 2, 2-Diphenyl-1-Picrylhydrazyl free radical scavenging activity (DPPH-RSA), and ferric reducing antioxidant potential (FRAP). All analyses were carried out in triplicate. The TPC for each component (mace, kernel, and pericarp) was determined following the Folin-Ciocalteu method as described by Velioglu et al. (1998). A sample extract of 0.2 mL was mixed with 1.5 mL of Folin-Ciocalteu reagent. The mixture was then allowed to stand at room temperature for 5 min, followed by adding 1.5 mL of 6% sodium carbonate solution. The resulting solution was incubated at room temperature for 90 min, and the absorbance was measured

at 725 nm using distilled water as a blank in a UV-visible spectrophotometer (Jenway UV-6300, UK). The results were expressed in gallic acid equivalents (GAE) in mg per gram of dry sample based on the prepared standard curve.

The TFC of different components was determined using an Aluminum Chloride assay through colorimetry according to Samatha et al. (2012). A total of 0.5 mL of sample extracts was mixed with 2 mL of distilled water and 0.15 mL of 5% Sodium Nitrite (w/v) and was allowed to stand for 6 min. To the resulting solution, 0.15 mL of 10% Aluminum Trichloride (w/v) was added, and the mixture was incubated for 6 min, followed by the addition of 2 mL of 4% Sodium Hydroxide (w/v). Finally, the volume was adjusted to 5 mL with distilled water and incubated for 15 min. Upon incubation, the absorbance was measured at 510 nm using a UV-visible spectrophotometer upon the colour change to pink. The TFC of the sample was expressed in mg of rutin equivalents (RE) per gram of dry sample based using a standard curve.

The DPPH-RSA of the mace, kernel, and peel extracts was assessed as described by Somaratne et al. (2017) and Premakumara et al. (2013), with slight modifications. A 300 μ L aliquot of each sample extract was mixed with 1.5 mL of DPPH solution. The mixture was incubated in the dark at room temperature for 40 min, and the absorbance was measured at 515 nm using a UV-visible spectrophotometer. The DPPH assay was performed using trolox to prepare the standard curve. Results were expressed as mg of trolox per gram of dry sample of nutmeg.

The total antioxidant potential of the sample was evaluated using the FRAP assay, as described by Benzie & Strain (1996) and Surveswaran et al. (2007). The FRAP reagent was prepared by combining 10 volumes of 300 mM acetate buffer (pH 3.6), 1 volume of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM hydrochloric acid, and 1 volume of 20 mM ferrous chloride. The mixture was diluted to one-third with 80% methanol and was pre-warmed to 37 °C. Subsequently, 3 mL of the FRAP reagent was added to 0.1 mL of the sample extract. The mixture was shaken and incubated at 37 °C for 8 min, and the absorbance was measured at 593 nm using a UV-visible spectrophotometer. The results were expressed as trolox equivalents in mg per gram of dry sample using a standard curve. Standard curves for each antioxidant assay were prepared using the specific wavelength designated for the respective test methods: TPC at 725 nm, TFC at 510 nm, DPPH-RSA at 515 nm, and FRAP at 593 nm.

Statistical analysis

The data were tested for normality using the Kolmogorov-Smirnov test ($p > 0.05$). The significance of the means of all parameters for mace, kernel, and peel was tested using a one-way ANOVA at $p < 0.05$, and mean separation was carried out using post-hoc Tukey HSD test using the statistical software SAS® on Demand for Academics (SAS 9.3M2).

RESULTS AND DISCUSSION

Proximate analysis of nutmeg mace, kernel, and pericarp

Proximate composition is an essential constituent for determining its end-use in the food industry, i.e., product development, quality control, and regulatory compliance. Table 1 shows the moisture, crude fat, crude fiber, ash, crude protein, and carbohydrate content of mace, kernel, and pericarp of nutmeg, which carry potential importance in the food industry. The results revealed a significant variation among mace, kernel, and pericarp with respect to proximate mean values of five nutmeg composites collected from three districts for all tested proximate components in mace, kernel, and pericarp of nutmeg.

According to Table 1, the crude fat content of mace, kernel, and pericarp was significantly ($p < 0.05$) different in each nutmeg component, where the kernel ($42.97 \pm 0.48\%$) carried the highest crude fat content, which is two-fold higher compared to mace ($24.57 \pm 0.51\%$) and approximately 21-fold more compared to that of pericarp ($2.05 \pm 0.02\%$). The studies done by Asika et al. (2016) and Okiki et al. (2023) reported a crude fat content in the Nigerian nutmeg kernel were 25.60% and 26.73%, respectively. Okonkwo (2014) reported a crude fat content of 13.34% following the same extraction method for samples collected from Nigeria; however, it is not specified if the reported value is for mace, kernel, or a mixture of both. In addition, a study conducted in Kerala, India reported a fat content of 31.37% for nutmeg kernels (Thomas & Krishnakumari, 2015). Compared to the previous studies, the current study reported a comparatively higher crude fat content in Sri Lankan nutmeg. Given the fact that both nutmeg essential oil and fixed oil (nutmeg butter) has a high market demand and fetch premium prices (Singh et al., 2003; Rema & Krishnamoorthy, 2012), the higher crude fat content carried by the Sri Lankan nutmeg indicates a potential advantage in the industry. To our knowledge, no studies have reported the crude fat content specifically for the mace and pericarp

Table 1: Comparison of proximate components (moisture, crude fat, crude fiber, ash, protein, and carbohydrate) in mace, kernel, and pericarp of Sri Lankan nutmeg.

Component	Moisture (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Protein (%)	Carbohydrate (%)
Mace	$56.89 \pm 0.57^{b*}$	24.57 ± 0.51^b	5.18 ± 0.26^b	0.36 ± 0.01^b	8.81 ± 0.26^a	5.97 ± 0.91^{ab}
Kernel	30.67 ± 1.78^c	42.97 ± 0.48^a	7.13 ± 0.22^a	0.21 ± 0.01^c	8.70 ± 0.25^a	11.42 ± 2.11^a
Pericarp	88.26 ± 0.77^a	2.05 ± 0.02^c	2.33 ± 0.06^c	0.50 ± 0.03^a	4.25 ± 0.38^b	4.97 ± 1.12^b

*Mean $\pm$ SD of all five composites collected from three districts. Values within a row followed by different simple letters are significantly different at $p < 0.05$.

of Sri Lankan nutmeg. Accordingly, the $24.56 \pm 0.50\%$ crude fat content reported in this study for mace is more or less similar to the crude fat content of the kernel reported by Asika et al. (2016) and Okiki et al. (2023). This indicates that both kernel and mace of Sri Lankan nutmeg have high potential for essential oil extraction. However, this study reported a very low pericarp crude fat content, and hence the pulp made out of the nutmeg pericarp could also be utilized as an ingredient in food processing industries such as in production of low fat jams, jelly and marmalades due to the high pectin content reported for the nutmeg pericarp (Latha et al., 2005 and Raj et al., 2016).

Considering the other proximate analysis constituents, a significant variation ($p < 0.05$) was observed in the ash content of kernel, mace, and pericarp of nutmeg; however, the reported values are in a low range for use in an industry. Similarly, significant variation ($p < 0.05$) was observed in the crude fiber content of kernel, mace, and pericarp of nutmeg, where the crude fiber content in the kernel was significantly higher ($p < 0.05$) compared to the crude fiber content of mace and pericarp. The reported crude fiber content of the kernel (7.13 ± 0.22) is around 2-7 times less than that reported elsewhere in the world (Okonkwo, 2014; Thomas & Krishnakumari, 2015; Asika et al., 2016; Okiki et al., 2023). Further, the moisture content was significantly ($p < 0.05$) different in mace, kernel, and pericarp of nutmeg, with the pericarp reporting the highest moisture content ($88.26 \pm 0.77\%$) and the kernel ($30.67 \pm 1.78\%$) the lowest as expected. The moisture content reported for the Sri Lankan nutmeg kernels was higher compared to studies conducted in Nigeria and India, where the moisture content ranged from 9.37 to 14.91%. The moisture content could well be dependent on the climatic conditions prevailing at the time of sample collection, where seeds harvested during a rainy spell may carry higher moisture content than those harvested during a dry spell. In Sri Lanka, the nutmeg kernels are mostly sun-dried by the farmers or at the collector level. When processing nutmeg kernels carrying higher moisture content, drying must be done for longer periods, and during that time, the kernels are more susceptible to the development of secondary pest and fungal infections. Therefore, proper drying techniques such as solar drying or mechanical drying must be established at the collectors' level to ensure better quality of the nutmeg products (Ratti, 2001; Kamiloglu et al., 2006; Zhang et al., 2006 and Janjai & Bala, 2012).

The crude protein content of mace (8.81%) was nearly equivalent to that of the kernel (8.70%) and was significantly higher ($p < 0.05$) than that of the peel, which contained approximately half the protein content of the mace and kernel (4.25%). Previous studies have reported varying crude protein contents for Nigerian nutmeg. Okiki et al. (2023) and Asika et al. (2016) documented crude protein levels of 18.67% and 7.02%, respectively, for the kernel. Okonkwo (2014) reported a crude protein content of 11.50%, although it was not specified whether this value pertained to the mace, kernel, or a mixture of both components. Variations observed in crude protein content compared to previous studies suggest potential variability in the nutritional composition of nutmeg due

to genetic, environmental, or post-harvest factors. These findings highlight the importance of region-specific studies to accurately characterize the nutritional properties of Sri Lankan nutmeg and its potential applications.

The current study showed no significant ($p < 0.05$) difference in carbohydrate content, where the values ranged from 4.97 to 11.42% for mace, kernel, and pericarp, in which the kernel exhibited the highest carbohydrate content. A previous study by Okiki et al. (2023) reported a carbohydrate content of 28.93% for the Nigerian nutmeg kernel. Additionally, Okonkwo (2014) documented a carbohydrate content of 41.57%; however, it was not specified which part of the nutmeg this value corresponded to.

This study revealed that the kernel displayed the highest crude fat content, while it carried the lowest moisture content. Conversely, the pericarp had the highest moisture content and lowest fat, positioning it as a candidate for low-fat product formulations, particularly where moisture retention is beneficial. Additionally, while the ash and crude fiber contents showed significant variation across parts, the levels remained within a lower range that may limit their industrial applications based on mineral or fiber content. Therefore, the study findings underscore the unique compositional profiles of nutmeg mace, kernel, and pericarp, suggesting specific advantages, highlighting potential uses in both food and non-food applications. The findings further emphasize the need for efficient processing and storage of nutmeg, highlighting the need for tailored postharvest handling techniques to maximize product quality and shelf life.

Antioxidant properties of nutmeg mace, kernel, and pericarp

Antioxidant potential can be measured by few different assays (TPC, TFC, DPPH-RSA, and FRAP) and these assays can be used to confirm the antioxidant content and capacity. These compounds have the potential to scavenge free radicals, chelate metal ions, or reduce oxidative damage. This study reported antioxidant properties of different parts of nutmeg (mace, kernel, and pericarp), which showed great potential to be used as a source of antioxidants in health and industrial applications. Determining antioxidant content in terms of TPC, TFC, DPPH radical scavenging activity and FRAP is crucial in evaluating its ability to neutralize free radicals that prevent oxidative stress. This highlights the potential health benefits of nutmeg and its applications in the industry. Colorimetric methods such as DPPH-RSA and FRAP assays are commonly used in measuring the direct involvement of extracts in enhancing primary antioxidant activity (Sulaiman & Ooi, 2012). The antioxidant activity was quantified based on trolox equivalents, providing a more direct, informative, and descriptive measure, compared to assays that indicate antioxidant activity by expressing it as a percentage absorbance reduction (Wong et al., 2006).

The values obtained for TPC and DPPH-RSA in mace were significantly different ($p < 0.05$) from the kernel and pericarp of nutmeg (Figure 1a and 1b). As illustrated in Figure 1a, the TPC was highest in mace (24.50 ± 3.31 mg GAE/g), exceeding the TPC of the kernel (13.87 ± 0.51 mg GAE/g)

and peel (8.093 ± 0.91 mg GAE/g) by a notable margin. Tan et al. (2013) reported more or less similar findings to the results of the current study in freeze-dried mace and kernel. Although adopting a different drying method, Assa et al. (2014) reported TPC for oven-dried mace and oven-dried kernels, which is approximately two-fold compared to the current study. Gupta et al. (2013) reported a lower TPC value for the oven-dried seed kernel samples at 40°C , which is considerably lower compared to all previous studies and the current study (21 times less). The air-dried samples in the current study showed lower total phenolic content (TPC) in mace, kernel, and peel than the freeze-dried samples reported by Tan et al. (2013). The variation in TPC results from differing temperatures in the drying process of plant materials. However, contradictory to the understanding that antioxidants are sensitive to exposure to high temperatures (Cisneros-Zvallos, 2003; Padua & Picha, 2007; and Réblová, 2012), the values reported by Assa et al. (2014) for oven-dried samples at 45°C resulted in higher TPC than the air-dried samples in the current study. This variation could be due to the differences in extraction and testing procedures used or due to the higher availability of antioxidants in the evaluated samples.

Additionally, for the aqueous extracts of nutmeg mace, kernel, and pericarp, Sulaiman & Ooi (2012) and Fernando & Senevirathne (2019) reported total polyphenolic content (TPC) values for mace ranging from 48.2 ± 1.2 to 83.56 ± 3.55 mg GAE/g and for the kernel from 46.3 ± 0.6 to 114.95 ± 4.3 mg GAE/g, which are higher than those observed in the present study. However, a direct comparison between these findings and the current study is not feasible, as the TPC values reported in the previous studies were based on the wet weight of the samples, whereas the present study expresses results in terms of the dry weight of

the samples. Despite the variations observed, the results of this study are in agreement with the previous findings (Tan et al., 2013; Assa et al., 2014), confirming that the mace sample contains a higher TPC than the kernel. However, the current study results on TPC of pericarp could not be compared with the TPC reported by Tan et al. (2013) and Assa et al. (2014), as these studies reported TPC separately for skin and pulp. Further, the results of this study could not be compared with the results reported by Sulaiman and Ooi (2012) and Fernando & Senevirathne (2019) due to differences in the results analysis.

According to Figure 1b, the DPPH-RSA value was found to be significantly ($p < 0.05$) higher in mace 33.09 ± 2.05 mg TE/g, compared to kernel 21.14 ± 3.67 mg TE/g and the pericarp content 16.96 ± 2.53 mg TE/g. The studies conducted by Fernando & Senevirathne (2019) and Sulaiman & Ooi (2012) revealed that the lowest IC_{50} and EC_{50} values for DPPH-RSA in nutmeg kernels, indicating that nutmeg kernels have higher antioxidant activity compared to mace. However, the findings of Fernando & Senevirathne (2019) and Sulaiman & Ooi (2012) could not be compared with the current study due to the differences in the drying techniques adopted and due to differences in the expression of the antioxidant capacity. The present study reports a significantly ($p < 0.05$) higher antioxidant activity of mace (33.09 ± 2.05 mgTE/1g) compared to kernel (21.14 ± 3.67 mgTE/1g) and pericarp (16.96 ± 2.53 mgTE/1g), and hence is not in agreement with the previous studies by Fernando & Senevirathne (2019) and Sulaiman & Ooi (2012) with respect to TPC. The DPPH-RSA assay has not been assayed for nutmeg in trolox equivalents; however, it has been conducted for other crops such as *Centella asiatica*, *Moringa pterygosperma*, *Piper betel* etc. (Wong et al., 2006). Interestingly, the DPPH-RSA reported for nutmeg

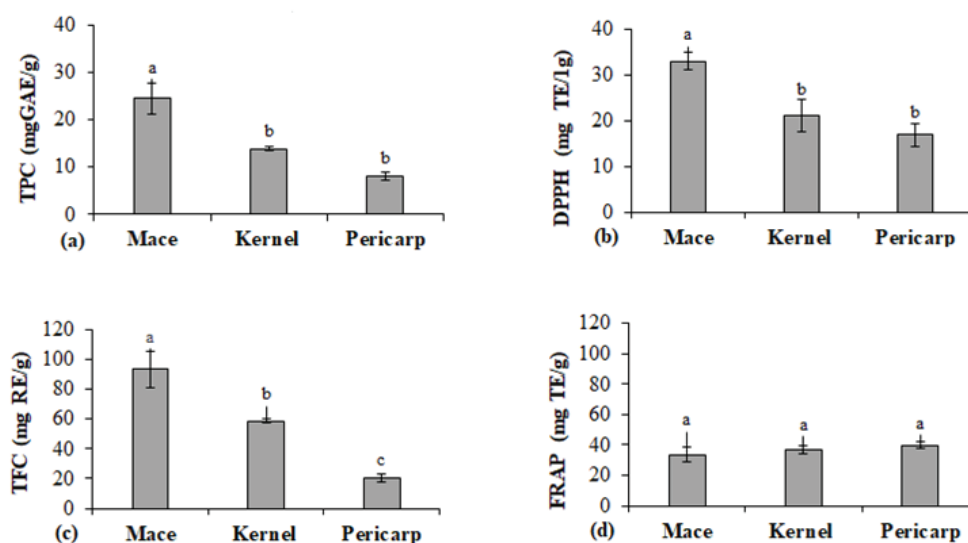


Figure 1: Variation in (a) Total phenolic content (TPC), (b) 2,2-Diphenyl-1-Picrylhydrazyl Radical scavenging activity (DPPH-RSA), (c) Total flavonoid content (TFC), (d) Ferric reducing antioxidant power (FRAP) of mace, kernel, and pericarp of Sri Lankan nutmeg. Values are expressed as mean $\pm$ SD ($n=15$). Different letters above the bars indicate significant differences among samples ($p < 0.05$).

in the current study in trolox equivalents is considerably higher compared to that of red rice bran (12.86-16.94 mgTE/g of sample) reported by Premakumara et al. (2013). Red rice bran is known for its marked antioxidant property, which opens up a potential market for nutmeg to be used as a key ingredient in developing functional foods, nutraceuticals, and cosmetics. The study conducted by Sathya et al. (2020) reported the DPPH radical scavenging activity in three chromatographically isolated compounds of acetate and methanol extracts of mace collected from the Kandy District in Sri Lanka. However, the study findings could not be compared with the current findings due to differences in methods adopted and differences in units. Three of the compounds identified in Sathya et al. (2020) exhibited DPPH radical scavenging activity, confirming the antioxidant potential of mace.

The TFC of nutmeg revealed a significant ($p < 0.05$) difference among mace, kernel and pericarp (Figure 1c). The TFC of mace was approximately five-fold higher than that of the pericarp, while the kernel was about three-fold higher than that of the pericarp. The TFC reported by Tan et al. (2013) for kernel ($1,345 \pm 18.26$ mg RE/g) and mace (245.50 ± 13.18 mg RE/g) were 22- and 2.5-fold higher than that reported in this study, respectively. This variation may be due to the differences in drying methods between the studies of Tan et al. (2013) and the present study.

The FRAP assay evaluates the ability of antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions, reflecting the electron-donating capacity of the compounds present (Benzie and Strain, 1996; Guo et al., 2003). In the current study, no significant differences ($p > 0.05$) were observed in the FRAP values among the mace, kernel, and pericarp of nutmeg, which ranged from 33.73 to 39.79 mg TE/g (Figure 1d).

In contrast, Sulaimann & Ooi (2012) reported EC_{50} values for the ferric reducing potential in the order of 28.8 ± 0.5 $\mu\text{g/mL}$ for mace, 33.1 ± 0.2 $\mu\text{g/mL}$ for kernel, and 158.2 ± 2.2 $\mu\text{g/mL}$ for pericarp, indicating the highest antioxidant potential in mace and the lowest in pericarp, an observation contrary to the findings of the current study. Similarly, Fernando & Senevirathne (2019) recorded the highest FRAP value for mace (178.53 ± 1.82 mg TE/g) and the lowest for pericarp (31.40 ± 1.34 mg TE/g), expressed in Trolox equivalents in the extract. These findings align with the trend observed by Sulaimann & Ooi (2012), but differ from the pattern reported in the present study.

The current study revealed notable differences in the antioxidant profiles across the different components of Sri Lankan nutmeg based on TPC, DPPH-RSA, TFC, and FRAP assays. Mace carried the highest TPC, TFC, and antioxidant activity in terms of DPPH-RSA compared to kernel and pericarp, indicating superiority in antioxidant properties. While the TFC of the kernel was not significantly ($p > 0.05$) different to mace, the kernel was found to exhibit antioxidant properties second to mace, but more than the pericarp with respect to TPC and DPPH-RSA. Based on the FRAP assay, all three components (mace, kernel, and pericarp) expressed similar ferric ion reducing ability. This uniformity indicates that each component

has a similar electron donating power to neutralize free radicals. These findings highlight dominance in phenolic and radical scavenging activity in mace, followed by the kernel, making each component a potential source for antioxidants. Though rated lowest in antioxidant potential, the pericarp may still contain specific compounds that make it appealing for certain industrial applications due to its reducing ability. Therefore, it holds promise for value-added uses as natural preservatives or nutraceuticals rich in bioactive compounds.

CONCLUSIONS

The mace, kernel, and pericarp of Sri Lankan nutmeg showed differences in moisture content, crude fat, crude fibre, and ash content. The crude fat content of the kernel was nearly 2-fold and 21-fold more compared to mace and pericarp, respectively. The mace, kernel, and pericarp of Sri Lankan nutmeg revealed differences in TPC, TFC, and DPPH-RSA; however, no significant difference was observed with respect to electron-donating ability in terms of the FRAP assay. Based on TPC, TFC, and DPPH-RSA, mace exhibited the best antioxidant properties, followed by the kernel. The pericarp, which is usually discarded as waste, exhibited the highest ferric-reducing antioxidant power. These results highlight the potential use of nutmeg mace, kernel, and pericarp for various industrial applications, other than their primary use as a spice.

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DECLARATION OF CONFLICT OF INTEREST

Authors declare no conflict of interest.

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AUTHOR CONTRIBUTION

Conceptualization: **D.J., R.P., S.R., P.H.**; Research Design and Methodology: **D.J., R.P., P.H., S.R.**; Funding acquisition and Project administration: **D.J., P.H.**; Supervision: **R.P., D.J., S.R., P.H.**; Sample collection: **D.J., P.H., S.P.**; Data collection and analysis: **S.P., S.R., P.H.**; Statistical analysis: **R.P., S.R., S.P.**; Manuscript writing: **S.P., S.R., P.H., D.J.**; Editing the manuscript: **R.P.**; All authors approved the final version of the manuscript.

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