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Proximate composition, total phenolic content, total flavonoid content, and nitric oxide radical scavenging ability of aqueous and methanolic extracts of red apple flesh

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ABSTRACT. Red apples are commonly consumed and widely available in global markets because of their sensory properties and sweet taste. This study determined the proximate composition, total flavonoid content (TFC), total phenolic content (TPC), and nitric oxide radical scavenging ability (NORSA) of red apple flesh (RAF). Ten fresh and ripe red apple fruits were collected, identified, authenticated, dried to a constant weight, macerated and decanted to obtain clear filtrates of the aqueous extract of red apple flesh (AERAF) and the 80% methanolic extract of red apple flesh (MERAF). Proximate analysis of RAF showed 86.665 ± 0.235% moisture, 3.235 ± 0.585% carbohydrate, 1.965 ± 0.015% protein, 5.500 ± 0.500% crude fibre, 0.835 ± 0.035% ash, and 1.800 ± 0.300% fat. AERAF had higher reported TPC (at 50 µg/mL) and TFC (at 25, 50 and 100 µg/mL). MERAF had higher reported TPC (at 25 and 100 µg/mL). Vitamin C exhibited higher nitric oxide radical scavenging activity than either extract. The reported assay responses increased with concentration for both extracts. Pearson correlation analysis identified a significant positive association between TPC and NORSA for AERAF ($r = 0.9180$, $p = 0.0279$); whereas the other reported correlations were not statistically significant. These findings provide preliminary, sample-specific information on the proximate composition, total phenolic contents, total flavonoid contents, and nitric oxide radical scavenging ability of red apple flesh extracts. Further research is required to characterise individual phytochemicals and evaluate the extracts using complementary chemical and biological assays to validate their potential food or nutraceutical applications.

Keywords: Total flavonoid contents, NO radical scavenging, total phenolic contents, Red apple flesh, Solvent-based extraction

1. INTRODUCTION

Apples (*Malus ?5* Borkh.) are widely consumed fruits and important dietary sources of phenolic compounds, including flavan-3-ols, flavonols, dihydrochalcones, and hydroxycinnamic acids (McGhie et al., 2005; Ferreira et al., 2024; Loy et al., 2025). These compounds have attracted scientific interest because of their chemical properties and their potential contribution to the antioxidant-related properties of apple-derived products (Ferreira et al., 2024; Loy et al., 2025). However, the composition and concentration of apple phenolics vary with cultivar, growing environment, fruit maturity, storage conditions, and processing methods (Kschonsek et al., 2018; Loy et al., 2025). Findings obtained from one cultivar or production context therefore cannot automatically be generalised to all apples. Phenolic compounds are also distributed unevenly among apple tissues. The peel generally contains higher

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concentrations of certain phenolic constituents than the flesh, although apple flesh also contains measurable quantities of these compounds (Tsao et al., 2003). These differences support investigating apple flesh as a distinct plant material (Asma et al., 2023) rather than assuming that its phytochemical composition and antioxidant-related properties are equivalent to those of the peel or whole fruit (McGhie et al., 2005; Manzoor et al., 2012).

Proximate composition analysis provides information about the major nutritional constituents of a sample, commonly including moisture, ash, crude protein, crude fat, crude fibre, and carbohydrate, depending on the analytical procedures employed. These measurements complement phytochemical investigations by providing a broader description of the material under investigation. Nevertheless, the nutritional composition and phenolic profile of apples can vary with cultivar and other biological and environmental factors (Loy et al., 2025). Therefore, determining proximate composition alongside total phenolic content (TPC), total flavonoid content (TFC), and the response in a nitric oxide-related assay can provide complementary information about the nutritional composition and selected *in vitro* chemical properties of red apple flesh.

The extraction of phenolic compounds depends on factors such as solvent polarity, solubility, extraction time, temperature, and the characteristics of the plant matrix (Babbar et al., 2014; Acquavia et al., 2021). Methanol and aqueous methanol are commonly used in phytochemical investigations because solvent composition influences the recovery of different classes of phenolic compounds, while water provides an alternative medium for extracting water-soluble constituents (Prior et al., 2005; Acquavia et al., 2021). However, extraction efficiency depends on the compounds of interest and the conditions employed; neither water nor methanol can be assumed to be universally superior for all plant matrices and phytochemical classes. Furthermore, a higher measured concentration of phenolic or flavonoid equivalents in an extract does not identify the individual compounds responsible for the result or, by itself, establish greater biological efficacy (Kschonsek et al., 2018; Acquavia et al., 2021). Therefore, comparing aqueous and 80% methanolic extracts under defined experimental conditions is useful for assessing their relative performance with respect to the particular apple flesh sample investigated (Kurćubić et al., 2025).

TPC and TFC assays provide estimates of the compounds that respond under the respective analytical conditions; they do not independently establish the identity or concentration of every individual phenolic or flavonoid compound present. Consequently, differences in these measurements between extracts should be interpreted as differences in assay responses rather than definitive evidence of the recovery of particular compounds. Similarly, antioxidant-related assays measure specific chemical responses under defined experimental conditions and should not be treated as interchangeable measures of overall antioxidant activity (Prior et al., 2005; Acquavia et al., 2021). In this study, nitric oxide-related activity was assessed using the sodium nitroprusside-Griess assay, which estimates nitrite-related signal associated with nitric oxide generation. Interpretation of this assay requires an appropriate control-based calculation and consideration of sample-related interference. However, the resulting measurements should be interpreted within the limits of this particular *in vitro* assay rather than as comprehensive evidence of antioxidant effects in biological systems. Any observed relationship between TPC, TFC, and the assay response should likewise be interpreted as an association, not proof that the measured phenolic or flavonoid constituents

directly caused the observed response.

A comparison of aqueous and 80% methanolic extracts can provide preliminary information to guide further investigations of red apple flesh and the selection of extraction conditions for subsequent phytochemical analyses. Such information may support more detailed studies involving the identification of individual phenolic compounds, evaluation of additional extraction solvents, and assessment using complementary antioxidant assays. However, potential food or nutraceutical applications require further investigation of extract composition, residual solvents, safety, bioaccessibility, and biological efficacy (Asma et al., 2023; Kurćubić et al., 2025). The analytical measurements reported in this study alone cannot establish therapeutic benefits, suitability for clinical use, or the effectiveness of red apple flesh in preventing or treating disease (Acquavia et al., 2021; Loy et al., 2025).

Therefore, this study aimed to determine the proximate composition of red apple flesh and compare the TPC, TFC, and response in a nitric oxide radical scavenging assay of its aqueous and 80% methanolic extracts. Specifically, the study sought to: (i) determine the proximate composition of red apple flesh; (ii) determine and compare the TPC of the aqueous and 80% methanolic extracts; (iii) determine and compare the TFC of the two extracts; (iv) evaluate and compare their responses in the sodium nitroprusside-Griess assay using an appropriate control-based calculation; and (v) examine the relationships between TPC, TFC, and the measured assay response.

2. METHODOLOGY

2.1. Reagents and chemicals

Chemicals and reagents used in the research were of analytical grade. Folin-Ciocalteu reagent, Griess reagent, and ascorbic acid were acquired from Sigma Aldrich Chemicals Co., USA. Potassium ferricyanide, sodium hydroxide, sodium nitroprusside, and sodium phosphate were bought from Randox Laboratories Ltd. (Crumlin, UK). The remaining chemicals were purchased from reputable suppliers.

2.2. Plant collection, authentication and extraction

The plant material used in this investigation was red apple (*Malus domestica*). Ten (10) viable, ripe, and fresh red apples were purchased from markets in Ogun State, Nigeria. The red apples were identified and authenticated at the Herbarium Laboratory, Department of Forestry and Wildlife Management, College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria and ascribed a voucher number UAHA/FWM/025/05/002. The exact cultivar of the red apples was identified as Red Delicious. The fruits were meticulously gathered, cleaned under running water, and rinsed with distilled water.

2.3. Aqueous and methanolic extraction of *Malus domestica*

The edible red apple flesh (RAF) was cut into tiny pieces after the edible flesh was separated from the peels. The flesh from the ten fruits was pooled into one composite sample and cut into small pieces. The tiny pieces were dried at 60 ^{o}C “using a Bosch food dehydrator (BS-6608)” until a consistent weight was obtained for maceration. The dried samples were ground into a coarse powder and stored in a tightly sealed container. A weighed portion of dried apple-flesh powder (200 g) was mixed with 500 mL of distilled water to give a solid-to-solvent ratio of 0.4 g:1 mL (w/v) (Azwanida, 2015). The mixture was macerated for 72 h at 25 ^{o}C, protected from light, and filtered through a

muslin cloth (0.1 mm pore size). The filtrate was concentrated using a rotary evaporator (Rotavap 110) at 40°C. The aqueous extract of red apple flesh (AERAF) was weighed and stored in a sealed, dry container until further use. The methanolic extraction was performed using 80% (v/v) methanol, with the same sample mass, solvent volume, extraction time, and processing conditions as the aqueous extract. The methanolic extract of red apple flesh (MERAF) was also weighed and stored in a sealed, dry container until further use. Extraction yield was calculated as: extraction yield (%) = (mass of dried extract / mass of dried starting powder) × 100.

2.4. Determination of moisture content

Moisture content was determined by drying a fresh RAF sample to constant mass using a hot-air oven method (AOAC, 2005). A 2 g RAF sample was weighed into a preweighed crucible. The crucible had been dehydrated at 103 °C for 3 hours in an oven. After the crucible and its contents had cooled in a desiccator, they were weighed. The loss in mass was recorded.

2.5. Determination of ash content

The AOAC (2010) was utilised for measuring the level of ash. A 2 g RAF sample was placed in a crucible and heated in a muffle furnace and heated to 550 °C until the contents became whitish or greyish. After cooling in a desiccator, the sample was weighed.

2.6. Determination of fat content

Fat content was determined in accordance with AOAC (2005). For Soxhlet extraction, 2 g of RAF was measured into a clean round-bottomed flask. The samples were then wrapped in a porous substance. The round-bottomed flask was then filled with 500 mL of petroleum ether and heated for four hours. Thereafter, the apparatus was cooled for four hours. The resulting mixtures were transferred, and the solvents were evaporated to leave a layer of fat.

2.7. Measuring the protein content

The AOAC (2005) was used to determine the level of nitrogen (N) in RAF based on the principle that the total nitrogen content of the sample is multiplied by a factor gives crude protein.

The equation used to determine the nitrogen constituents in RAF is provided below:

$$\% \text{ Nitrogen} = \frac{volume\ of\ sulphuric\ acid\ (mL) \times normality\ of\ sulphuric\ acid \times 1.4007}{Sample\ weight\ (g)}$$

Afterwards, the % nitrogen value derived was multiplied with 6.25 (protein factor) to obtain the protein content in RAF. % Crude protein = % Nitrogen x 6.25

2.8. Measuring the crude fibre content

This was done by AOAC (2005). Briefly, dried RAF (5 g) and 100 mL of trichloroacetic acid (TCA) were placed into an Erlenmeyer flask (500 mL). The contents were heated for 40 min, followed by slight cooling and filtration using a "No. 4" Whatman paper. Thereafter, the residue was transferred into a porcelain dish. The residue was dried

at 105 °C overnight. The dried product was placed in a desiccator and weighed as W1. The dried product was burnt for six hours at 500 °C in a muffle furnace, allowed to cool and reweighed as W2.

2.9. Determination of carbohydrate

Carbohydrate composition in RAF was measured by difference as

$$\% \text{ carbohydrate} = 100\% - (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Fibre} + \text{Ash})\%$$

2.10. Measuring the Total Phenolic Content (TPC)

Using the Folin-Ciocalteu method, TPC of AERAF and MERAF was estimated by spectrophotometry (Singleton et al., 1999). Gallic acid was used to plot a standard calibration curve. TPC of MERAF and AERAF was described as milligram gallic acid equivalents per gram dry weight (mg GAE/g DW). Gallic acid calibration curve was prepared under the same assay conditions as the samples. Sample absorbance was converted to equivalent concentration using the relevant calibration equation, corrected for the dilution factor, and normalized to the accurately recorded mass of dry weight. Values outside the validated calibration range were diluted and re-assayed rather than extrapolated. If they still remained outside the validated calibration range after dilution, the values were regarded as below the limit of detection (below LOD). The analysis was done using three technical replicates.

$$\text{TPC in mg GAE/g DW} = \frac{DF \times V \times C}{m}$$

Where: DF = Dilution Factor

C = GAE concentration expressed in milligram per milliliter

V = Entire volume of MERAF or AERAF expressed in milliliter

m = Mass of MERAF or AERAF expressed in gram

2.11. Measuring the Total Flavonoid Content (TFC)

The TFC of MERAF and AERAF was determined using the aluminium chloride method described by Ordonez et al. (2006). About 20 µL of MERAF and AERAF was introduced into a 2% $AlCl_3.6H_2O$ solution. There was vigorous shaking of the mixture followed by subsequent dilution with distilled water till an entire/final volume of 10 mL was achieved. Absorbance was taken at 440 nm after incubation for 10 minutes. TFC was recorded in milligram quercetin equivalents per gram of dry weight (mg QE/g DW). Quercetin calibration curve was prepared under the same assay conditions as the samples. Sample absorbance was converted to equivalent concentration using the relevant calibration equation, corrected for the dilution factor, and normalized to the accurately recorded mass of dry weight. Values outside the validated calibration range were diluted and re-assayed rather than extrapolated. If they still remained outside the validated calibration range after dilution, the values were regarded as below the limit of detection (below LOD). The analysis was done using three technical replicates.

$$\text{TFC in mg QE/g DW} = \frac{DF \times V \times C}{m}$$

Where: DF = Dilution Factor

C = QE concentration expressed in milligram per milliliter

V = Entire volume of MERAF or AERAF expressed in milliliter

m = Mass of MERAF or AERAF expressed in gram

2.12. Nitric oxide radical (NO) scavenging assay (NORSA)

Nitric oxide radical (NO**)** produced from sodium nitroprusside (SNP) was estimated using the method described by Marcocci et al. (1994). Incubation of 5 mM SNP "in phosphate-buffered saline (pH 7.3)" and MERAF or AERAF was done for 180 min at 25°C. The NO produced reacted with oxygen to generate nitrite ion (NO_2^-) that were detected at intervals of 30 mins by mixing Griess reagent (0.1% naphthylethylenediaminedihydrochloride and 1% sulfanilamide in 5% phosphoric acid) with the incubation mixture (1.0 mL). Absorbance value was determined between 120 to 180 minutes of reaction at 546 nm. The sodium nitroprusside-Griess assay was used to measure nitrite formed under the assay conditions. Nitrite concentration is reported as an indirect assay endpoint and is not itself a percentage nitric oxide scavenging value. Thereafter, percentage inhibition of nitric oxide radical formation was calculated relative to the blank or control using the equation provided below. Vitamin C was included as a positive reference control and was processed under the same assay conditions. The analysis was done using three technical replicates.

Nitric oxide radical (NO) scavenging ability was calculated as

$$= \frac{Absorbance\ of\ control\ or\ blank - Absorbance\ of\ sample\ or\ extract}{Absorbance\ of\ control\ or\ blank} \times 100$$

2.13. Correlation of TPC and TFC with observed nitric oxide radical scavenging ability

Pearson correlation analytical approach was used to show the association between beneficial plant chemicals (TPC and TFC) in AERAF and MERAF and nitric oxide radical scavenging ability of AERAF and MERAF. Statistical analyses were performed using SPSS version 20.0. However, the findings from Pearson correlation were carefully interpreted due to limited sample sizes and limited number of extract concentrations.

2.14. Statistical Analysis

All experimental procedures were done using three technical replicates. Data were analysed using SPSS version 20.0. Results are shown as "mean ± standard error of the mean (SEM)". Two-way analysis of variance (ANOVA) was adopted for statistical comparisons "to evaluate the effects of extraction solvent type (distilled water versus 80% methanol) and varying concentration (5, 10, 25, 50 and 100 µg/mL) of the extracts (AERAF and MERAF)". Duncan's multiple range tests (DMRTs) were used to compare the level of significance (considered at $p < 0.05$) among or across groups.

3. RESULTS AND DISCUSSION

3.1. Proximate composition of red apple flesh

Table 1 shows the nutritional components of RAF important for understanding the nutritional profile of the fruit. Fresh weight of RAF is shown to be primarily composed of water (86.665%), with smaller amounts of ash (inorganic minerals), fat, protein, carbohydrate, and fibre. The proximate content of RAF was found to range between 0.835 ± 0.035% (for ash) and 86.665 ± 0.235% (for moisture). Moreover, the fat, protein, carbohydrate and crude fibre contents were observed to be 1.800 ± 0.300%, 1.965 ± 0.015%, 3.235 ± 0.585% and 5.500 ± 0.500%, respectively. The observed results reflect the role of apples as a hydrating, low-calorie fruit with moderate amounts of fibre and other nutrients beneficial for human health (Efimtseva and Chelpanova, 2020). However, variation in the nutritional components of RAF suggests some natural differences that can arise from harvest time, environmental conditions, and fruit maturity (Molina-Corral et al., 2021).

Table 1. Proximate composition of fresh weight of RAF.

Constituents	Proximate Analysis of Red apple flesh (%); n=3
Ash	0.835 ± 0.035
Moisture	86.665 ± 0.235
Fat	1.800 ± 0.300
Crude fibre	5.500 ± 0.500
Protein	1.965 ± 0.015
Carbohydrate	3.235 ± 0.585

Note: Constituent contents are expressed as percentages and presented as mean ± SEM from three technical replicates.

3.2. TPC and TFC of MERAF and AERAF

Quantitative analyses of MERAF and AERAF for TPC and TFC are provided in Table 2. TFC and TPC (of MERAF and AERAF) were expressed in mg QE/g DW and mg GAE/g DW, respectively. TPC differed in MERAF and AERAF, with values from 39.077 ± 0.581 mg GAE per 1 g DW (25 μg/mL) to 69.923 ± 0.291 mg GAE per 1 g DW (100 μg/mL) and from 0.692 ± 0.277 mg GAE per 1 g DW (5 μg/mL) to 69.051 ± 1.096 mg GAE per 1 g DW (100 μg/mL), respectively. TFC was also observed to vary in MERAF and AERAF, with values from 4.984 ± 0.067 mg QE per 1 g DW (25 μg/mL) to 46.797 ± 0.727 mg QE per 1 g DW (100 μg/mL) and from 7.496 ± 1.075 mg QE per 1 g DW (25 μg/mL) to 62.033 ± 0.512 mg QE per 1 g DW (100 μg/mL), respectively. However, TFC for MERAF and AERAF (at 5 μg/mL and at 10 μg/mL), as well as TPC for MERAF (at 5 μg/mL and 10 μg/mL) were observed to be less than zero; hence, they were represented as Below the Limit of Detection (Below the LOD). Moreover, TPC and TFC in MERAF and AERAF were noticed to undergo dose-dependent increase because as the concentration of MERAF and AERAF increased, total phenolics and flavonoids were increased.

Table 2. TFC and TPC of MERAF and AERAF.

	Total flavonoid content (mg QE/g DW)		Total phenolic content (mg GAE/g DW)	
Concentration	MERAF	AERAF	MERAF	AERAF
5 μg/mL	Below the LOD	Below the LOD	Below the LOD	0.692 ± 0.277[a]

10 µg/mL	Below the LOD	Below the LOD	Below the LOD	1.769 ± 0.464^{a}
25 µg/mL	4.984 ± 0.067^{a}	7.496 ± 1.075^{a}	39.077 ± 0.581^{c}	30.000 ± 2.021^{b}
50 µg/mL	40.244 ± 1.128^{b}	41.081 ± 3.127^{b}	46.179 ± 0.185^{d}	50.487 ± 0.530^{e}
100 µg/mL	46.797 ± 0.727^{c}	62.033 ± 0.512^{d}	69.923 ± 0.291^{f}	69.051 ± 1.096^{f}

Note: TFC and TPC are presented as mean ± SEM (n = 3 representing three technical replicates) with unit as mg QE/g DW and mg GAE/g DW, respectively. Below the LOD - Below the Limit of Detection. Values along or across columns with different superscripts are considered significant at $p < 0.05$.

The observable variations from Table 2 suggest that the concentration of phenolics in MERAF and AERAF is significantly impacted by both solvents and their extracts' concentration. At 25 µg/mL, MERAF revealed significantly ($p < 0.05$) higher TPC relative to AERAF (25 µg/mL), whereas AERAF (50 µg/mL) had significantly ($p < 0.05$) higher TPC relative to 50 µg/mL MERAF. The observation at 25 µg/mL can be attributed to the higher efficiency of methanol as a solvent for extracting phenolic compounds (Babbar et al., 2014). Likewise, our finding is consistent with expectations, as higher concentrations of the extract typically result in more bioactive compounds being available for measurement (Babbar et al., 2014; Dhawan and Gupta, 2017; Rajhard et al., 2021).

Moreover, as observed, only 100 µg/mL AERAF reflected a significantly ($p < 0.05$) higher TFC relative to 100 µg/mL MERAF, which may indicate that water is more efficient at extracting flavonoids at 100 µg/mL or that specific flavonoid compounds are more readily extracted in aqueous solutions (Table 2). Thus, the effectiveness of methanol in solubilising a broad range of hydrophobic and hydrophilic phenolic compounds in RAF is higher than that of distilled water due to its polarity (Rajhard et al., 2021), which is why it is commonly used in phytochemical extractions (Dhawan and Gupta, 2017). However, our observation is also that at 50 µg/mL, water becomes more efficient at extracting phenolic compounds or that a certain phenolic compound is more abundant or more easily extracted from the fruit when using water as the solvent.

The higher total phenolic content observed in MERAF compared with AERAF at several concentrations may be linked to the superior extraction efficiency of methanol toward phenolics of intermediate polarity. Methanol has been widely reported to solubilise both hydrophilic and moderately hydrophobic phenolic constituents more effectively than water, thereby enhancing the collection of bioactive compounds such as hydroxycinnamic acids, flavan-3-ols, flavonols, and dihydrochalcones (McGhie et al., 2005; Oyenihi et al., 2022) from plant matrices (Babbar et al., 2014; Dhawan and Gupta, 2017; Rajhard et al., 2021).

3.3. Nitric oxide radical scavenging ability of AERAF and MERAF

The results of Nitric oxide radical scavenging ability are highlighted in Table 3 (an average of three technical replicates). The nitric oxide radical scavenging activity of MERAF and AERAF is expressed as percentage inhibition relative to the control, based on the indirect measurement of nitrite formed during SNP decomposition at 25 °C. The concentration of nitrite produced indirectly indicates the nitric oxide radical scavenging ability of AERAF and MERAF. The nitric oxide radical scavenging ability of AERAF and MERAF was observed to increase

as extract concentration increased.

Table 3. Nitric Oxide Radical Scavenging Assay (NORSA) for MERAF and AERAF.

Concentration	Nitric Oxide Radical Scavenging Ability (%)		
	MERAF	**AERAF**	**Vitamin C**
5 µg/mL	14.020 ± 0.176^{a}	14.166 ± 0.791^{a}	28.660 ± 0.459^{e}
10 µg/mL	25.027 ± 0.477^{c}	21.997 ± 0.744^{b}	29.701 ± 0.263^{e}
25 µg/mL	26.780 ± 0.425^{d}	22.782 ± 0.084^{b}	33.279 ± 0.066^{f}
50 µg/mL	32.384 ± 0.400^{f}	29.883 ± 1.430^{e}	36.948 ± 0.256^{g}
100 µg/mL	35.798 ± 0.132^{g}	32.348 ± 0.675^{f}	44.998 ± 0.102^{h}

Note: Nitric Oxide Radical Scavenging Ability are presented as mean ± SEM (n = 3 representing three technical replicates) with unit as percentage (%). Values along or across columns with different superscripts are considered significant at $p < 0.05$.

As reported in Table 3, the NO radical scavenging abilities of MERAF and AERAF are denoted in percentage (%). The NO radical produced from SNP alters the arrangement and functionality of several cellular components (Oyenihi et al., 2022). When produced in excess, NO interacts with superoxide anions ($^{\bullet}O_2^-$) to form peroxynitrite ($ONOO^-$), a potent oxidative molecule that can cause significant cellular damage (Barnabas et al., 2023). Therefore, NO radical scavenging ability is an important measure of a substance's potential to mitigate oxidative damage and protect cellular integrity from harmful radicals.

This research reports that as the concentration of extract increases, nitric oxide radical scavenging ability increased and vice versa. It is proposed that an increase in the presence of antioxidant compounds within extracts contributed to an increase in their capacity to scavenge NO radicals, which is a documented phenomenon for many plant-based antioxidants (Oyenihi et al., 2022). MERAF and AERAF (100 µg/mL) scavenged NO radical most efficiently, meaning they would produce lower nitrite concentrations, followed by 50 µg/mL, 25 µg/mL, and 10 µg/mL (Table 3). The lowest nitric oxide radical scavenging ability of MERAF and AERAF was noticed at 5 µg/mL. These findings indicate that red apple flesh extracts exhibit measurable nitric oxide radical scavenging activity under the assay conditions (Oyenihi et al., 2022). These findings indicate that red apple flesh extracts possess measurable nitric oxide radical scavenging ability under the sodium nitroprusside-Griess assay.

3.4. Pearson correlation analysis

Pearson correlation analysis (Table 4) demonstrated very strong positive association among TPC/TFC and nitric oxide radical scavenging ability (NORSA) of MERAF and AERAF. TPC showed strong positive correlation with NORSA in MERAF and AERAF as 0.9040 and 0.9180, respectively, although the observation was only significant (at $p < 0.05$) in AERAF. The observation proposes that phenolic compounds in AERAF may contribute to the removal of nitric oxide radicals. Similarly, TFC showed strong positive association with NORSA in MERAF and AERAF as 0.9720 and 0.9900, respectively, although the two observations were not significant at $p < 0.05$.

Table 4. Pearson correlation analysis between beneficial plant chemicals (TPC and TFC) and nitric oxide radical scavenging ability (NORSA) of methanolic and aqueous extracts of red apple flesh.

Correlation	Sample	Approximate Pearson r value	p-value	Significant (at $p < 0.05$)	Interpretation
TPC vs NORSA	MERAF	0.9040	0.2809	No	Very strong positive
TPC vs NORSA	AERAF	0.9180	0.0279	Yes	Very strong positive
TFC vs NORSA	MERAF	0.9720	0.1516	No	Very strong positive
TFC vs NORSA	AERAF	0.9900	0.0890	No	Very strong positive

Note: Correlation between parameters was deemed statistically significant at $p < 0.05$. Strength of the relationship between parameters was interpreted as very strong positive ($r \geq 0.90$), strong positive ($0.70 \leq r < 0.90$), moderate positive ($0.40 \leq r < 0.70$), and weak positive l($r < 0.40$).

Similar observations to our findings from correlation analysis have been highlighted for apple-derived phytochemicals, where phenolic constituents were identified as the principal determinants of antioxidant activity and protective biological impact (McGhie et al., 2005; Opriş et al., 2022; Oyenihi et al., 2022). Phenolics such as caffeic acid and p-coumaric acid are reported to possess hydroxyl groups capable of donating hydrogen atoms to neutralise free radicals (Hernández-Rodríguez et al., 2019; Akbari et al., 2022). Our observation is also consistent with the understanding that many phenolic compounds exert antioxidant effects by single-electron transfer and hydrogen-atom transfer (Akbari et al., 2022).

In addition, flavonoids possess multiple hydroxyl groups that donate hydrogen atoms or electrons to reactive oxygen and nitrogen species, thereby interrupting oxidative chain reactions and stabilising free radicals (Hernández-Rodríguez et al., 2019; Akbari et al., 2022). Previous reports by Oyenihi et al. (2022) and Nuñez et al. (2025) describe flavonoids as important bioactive constituents responsible for the health-promoting effects of apples and other fruits. Flavonoids such as quercetin, rutin, and catechin are recognised as potent electron donors that stabilise reactive oxygen species and interrupt oxidative chain reactions (Hernández-Rodríguez et al., 2019; Akbari et al., 2022). By donating electrons to oxidising species, scavenging reactive oxygen and nitrogen radicals, and preventing radical propagation, phytochemicals may help maintain cellular redox balance and protect biomolecules from oxidative damage (Barnabas et al., 2023).

4. CONCLUSION

This study provides a preliminary comparison of aqueous and 80% methanolic preparations of Red Delicious apple flesh, using proximate measurements, total phenolic and flavonoid assays, as well as sodium nitroprusside-Griess assay (for nitric oxide scavenging ability). This study demonstrates that red apple flesh is a valuable source of nutrients with reported biological significance. Both methanolic (MERAF) and aqueous (AERAF) extracts exhibited appreciable total phenolic and flavonoid contents, which were present in a concentration-dependent manner. The observed differences between MERAF and AERAF highlight the influence of solvent polarity on the recovery of phenolic and flavonoid constituents from red apple flesh. Both MERAF and AERAF showed nitric

oxide radical scavenging abilities due to a possible contribution of phenolic and flavonoid compounds in the red apple flesh; however, the small number of technical replicates limits the strength of this inference. These findings should be regarded as preliminary analytical observations, not proof of health benefits, cellular protection, or suitability for nutraceutical use. Future studies should use independently sampled fruit batches, validated calibration curves, transparent extraction-yield calculations, control-corrected antioxidant assays, and compound-specific profiling.

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AUTHOR CONTRIBUTIONS

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