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Comparative Evaluation of Antioxidant Activity of Methanolic and Aqueous Extracts of *Malus Domestica* (Red Delicious Cultivar)

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About Article

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ABSTRACT

Oxidative stress, caused by an excess of reactive oxygen species, is also known to play a role in the development of Cardiovascular, Metabolic, and Neurodegenerative disorders. Phytochemical compounds from plants, and in particular phenolics, are known to work as natural antioxidants in the diet that could limit oxidative stress. *Malus domestica* (Red Delicious cultivar) is very common and rich in polyphenols and Vitamin C. Hence, it could be an apt source for natural antioxidants. This study evaluated and compared the in-vitro antioxidant activities of methanolic and aqueous flesh extracts of *M. domestica*. Fruits were collected from Shagamu, Ogun State, Nigeria. Flesh extraction from the apple was performed using both methanol and water, and the concentration tested was 5-100 µg/mL. All the assays were done thrice, and the data was presented as mean ± SEM using one-way ANOVA analysis with $p < 0.05$. In both fractions, antioxidant activity increased with concentration. In the FRAP assay, the methanolic extract exhibited significantly higher ferric-reducing power than the aqueous extract at all tested concentrations, with values of 25.315 ± 0.149 mg TE/g and 18.619 ± 0.211 mg TE/g, respectively, at 100 µg/mL. In the ABTS⁺ assay, the aqueous extract showed higher activity at 5 µg/mL (16.334 ± 0.045 mg TE/g) compared to the methanolic extract (8.821 ± 0.094 mg TE/g); however, the methanolic extract demonstrated greater activity at higher concentrations, reaching 44.923 ± 0.866 mg TE/g at 100 µg/mL compared to 27.951 ± 0.907 mg TE/g for the aqueous extract. These findings confirm the presence of measurable in vitro antioxidant activity in *M. domestica* flesh and highlight the influence of extraction solvent on antioxidant capacity.

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1. INTRODUCTION

1.1. Reactive oxygen species and oxidative stress

Aerobic organisms produce ROS as by-products of normal metabolic processes, including superoxide ($O_2^{\bullet-}$), hydroxyl radicals ($\bullet OH$), and hydrogen peroxide (H_2O_2) (Sies & Jones, 2020). These ROS at low concentrations serve as important signaling molecules, modulating various intracellular pathways that control cellular homeostasis. However, with excess production of ROS in that cell's environment it creates an "oxidative stress," causing damage to the DNA, lipids, and proteins of the cell. This oxidative stress has been linked to many chronic diseases, including atherosclerosis, cancer and neurodegenerative disorders (Liguori *et al.*, 2018).

Mitochondria constitute a major intracellular site of production of reactive oxygen species (ROS), which is attributed mainly to the leakage of electrons mainly from complexes I and III of the mitochondrial respiratory chain, giving rise to the production of superoxide radicals ($O_2^{\bullet-}$). The superoxide is then converted into hydrogen peroxide (H_2O_2), which can produce highly reactive hydroxyl radicals ($\bullet OH$) through metal-catalyzed reactions (Sies *et al.*, 2017). In addition to generating reactive oxygen species (ROS) via mitochondria, several other cellular processes utilize enzymes to produce ROS. Among these are NADPH oxidases that intentionally produce ROS as part of their cellular signalling and for the role of defense within the immune system and Xanthine oxidase which also produces ROS while breaking down purines. Besides these sources of ROS produced by the body's own enzymes, there are a variety of outside influences that also create oxidative stress. Examples of this include: ultraviolet (UV) radiation and many types of pollutants, including cigarette smoke, which add additional oxidative stress on the cellular level (Sies & Jones, 2020). Other poor lifestyle choices, such as eating too many processed foods and emotionally taxing lives due to constant psychological stressors also create free radicals and weaken the body's natural defenses against free radicals. The combination of all of these things creates an imbalance that increases oxidative stress and increases cellular and tissue injury.

1.2. Endogenous and dietary antioxidant defense systems

Despite living organisms constantly producing reactive oxygen species (ROS), through evolution they have developed highly advanced antioxidant protection mechanisms to prevent damage created by ROS and thereby preserve the balance of oxidation and reduction. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) serve as the primary defense mechanism against ROS. These types of proteins belong to the first line of defence, and thus serve as biocatalysts to clear up the ROS found intracellularly. These types of proteins complete numerous redox reaction types to clear ROS through dismutation, reduction, and decomposition reactions, where the end product is to create non-toxic and less reactive compounds from ROS by way of a biocatalytic process (Sies *et al.*, 2017; Sies *et al.*, 2022). In addition to the antioxidant systems that we have already discussed and described previously, there is a great deal of research that demonstrates the beneficial effects of dietary antioxidants in terms of preventing or modifying the effects of

oxidative stress-related diseases through the use of antioxidants. Vitamin E (α -tocopherol), Vitamin C (ascorbic acid), carotenoids, phenolic acids, and flavonoids are a few of the most common dietary sources of antioxidants (Liguori *et al.*, 2018; Durazzo *et al.*, 2019). Eating foods that have high amounts of antioxidants on a regular basis has been consistently correlated to having lower levels of oxidative stress, and thus lead to improved physiological function and better overall health outcomes (Durazzo *et al.*, 2019).

1.3. Synthetic versus natural antioxidants

The use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butyl hydroquinone (TBHQ), and propyl gallate (PG) in the food industry to slow down lipid peroxidation and preserve shelf life has long been in use (Lourenço *et al.*, 2019). Although such substances are proven to be effective and economically viable, there are growing concerns and reservations being raised over their possible hepatotoxicity, hormone-disrupting effects, and carcinogenic effects exhibited at high doses being used in foods (Carocho *et al.*, 2018). Thus, the acceptable levels allowed by the regulations are being strictly enforced, and there has been a significant shift in the preference of consumers to look to more natural substitutes instead (Shahidi & Peng, 2025). Such developments in the food industry have therefore initiated efforts to seek out plant sources of antioxidants, such as polyphenols and other bioactive compounds that are more consumer-acceptable options for both health promotion and food preservation (Lourenço *et al.*, 2019).

1.4. *Malus domestica* as a source of dietary antioxidants

Bioactive compounds that exhibit strong antioxidant effects are often found in a large variety of fruits consumed within our diets. *Malus domestica* is one of the most frequently eaten fruits around the world and serves as an excellent source of phenolics and ascorbic acid for human consumption. There are several classes of bioactive compounds within the polyphenolic profile of *M. domestica*, including flavonoids such as quercetin glycosides, catechin, epicatechin, and phloridzin; hydroxycinnamic acids such as chlorogenic acid; and oligomeric and polymeric procyanidins (Geană *et al.*, 2021). Such phytochemicals may express effective radical-scavenging activity, metal-chelating capacity, and enzyme-modulating effects. Specifically, about two-thirds of *M. domestica* phenolics are located in the peel, which strongly underscores the nutritional relevance of whole-fruit consumption.

In general, epidemiological studies, as well as experimental investigations, have indicated that regular consumption of *M. domestica* has been linked to numerous positive health effects. Greater intakes of flavonoids from *M. domestica* have been associated with higher reduced levels of adiposity, lower type 2 diabetes risks, and improved metabolic function, possibly by a coordinated interaction among dietary fiber, polyphenolic compounds derived from the apple, and gut microbial metabolism (Bondonno *et al.*, 2018; Koutsos *et al.*, 2015; Dehghani Zahedani *et al.*, 2021). In addition, *M. domestica* consumption may exert cardioprotective effects by lowering levels of circulating low-density lipoprotein, preventing



the oxidation of LDL, enhancing endothelial function, and modulating inflammatory markers independently of changes in cardiovascular risk factors.

Emerging evidence also indicate the polyphenolic compounds present in *M. domestica* may help in reducing the level of oxidative stress and neuroinflammation within the nervous tissues. This may play a key role in the prevention of neurodegenerative disorders. Altogether, the above findings place *M. domestica* in a corner where it is not only a common fruit but a functional food as well.

1.5. Measurement of antioxidant capacity: Assay principles

Various test methods can be used to determine the true in-vitro antioxidant potential of natural products. The in-vitro tests that primarily utilize either hydrogen atom transfer (HAT) or electron transfer (ET) mechanisms are used for determining how well an antioxidant works and how these actions are accomplished. HAT tests, such as oxygen radical absorbance capacity (ORAC), are used to assess the capacity of antioxidants to donate a hydrogen atom to a free radical. ET tests are used to determine how well an antioxidant has the ability to project electrons into other chemical species, or in other words, how well that antioxidant has the ability to reduce or scavenge a free radical such as Trolox equivalent antioxidant capacity, ferric reducing antioxidant power, and the 2,2'-diphenyl-1-picrylhydrazyl [DPPH] radical scavenging assay. The methodologies described above have different chemical focuses and may be sensitive to various groups of compounds. For a more accurate understanding of the antioxidant capacity of a complex mixture of phytochemicals, it is recommended that a combination of both types of methods be utilized when performing a comprehensive antioxidant capacity test (Kotha *et al.*, 2022).

Of the various methods based on ET, the most commonly used antioxidant assays based on plant materials have been the ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). This technique quantifies the antioxidant's ability to reduce the blue-green radical cation, the ABTS^{•+} radical, to the neutral, colorless compound. The decrease in absorbance at a concentration-dependent wavelength of 734 nm upon the reduction of the radical is measured and quantified in units of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

On the other hand, the FRAP test relies on a complementary principle that estimates the reducing abilities of antioxidants via the reduction of ferric ions. In this case, the test assesses the reduction of the ferric tripyridyltriazine complex (Fe³⁺-TPTZ) to the blue-colored ferrous form (Fe²⁺-TPTZ) at an acidic pH of 3.6 (Christodoulou *et al.*, 2022). It is evident that the absorbance value at 593nm is directly proportional to the ferric reducing abilities of the sample and is also quantified in terms of Trolox and ascorbic acid equivalent values. When combined, the ABTS and FRAP tests offers an effective and reliable means of assessing the antioxidant property of the *M. domestica* and plant-based systems.

1.6. Research gap and study objective

Although there has been a substantial amount of work done

on the phenolic compounds and antioxidant activity present in *M. domestica*, there still exist several gaps when it comes to regional and particular variety differences. The specific variety of Red Delicious grown in Nigeria has been little studied, and there has been a marked lack of comparisons between aqueous and methanolic extraction methods using ABTS and FRAP on *M. domestica*.

At the same time, consumer awareness together with increasing scrutiny of regulatory authorities have raised the stakes for demand for natural antioxidants that are safe, effective, and of food-grade quality to replace synthetic compounds. This point nicely illustrates the convergence between scientific inquiry and the needs of society and entails the importance of scientifically establishing the value of the local varieties of *M. domestica* for antioxidants and the role of extraction conditions in this regard.

1.7. Novelty and regional significance

Despite the extensive global literature on *M. domestica* antioxidant properties, significant knowledge gaps remain regarding cultivar-specific and region-specific variations in antioxidant capacity. The Red Delicious cultivar grown in Nigeria has received minimal scientific attention, with no published comparative studies evaluating solvent-dependent extraction efficiency using both FRAP and ABTS methodologies in parallel. This study addresses these gaps by providing the first systematic assessment of antioxidant activity in Nigerian-grown Red Delicious *M. domestica* flesh extracts using complementary electron transfer-based assays. The regional significance of this work extends beyond basic characterization: Nigeria's diverse agro-ecological zones and cultivation practices may influence phytochemical composition in ways distinct from temperate-climate production systems documented in existing literature. By generating region-specific baseline data, this study supports evidence-based development of local value-added products, informs optimization of post-harvest processing protocols for Nigerian apple production, and contributes to the broader scientific understanding of how geographic origin and cultivar selection interact to determine the functional properties of widely consumed fruits. In addition, the direct comparison of aqueous and methanolic systems for extraction purposes can be of practical importance in the choice of an appropriate extraction solvent depending on the envisaged applications in food preservation, nutraceutical design, and functional food production for the Nigerian and West African communities.

2. LITERATURE REVIEW

2.1. Solvent extraction effects on apple antioxidants

The recovery and bioactivity of antioxidants derived from *M. domestica* have remarkably been influenced by the methodologies applied, with past research confirming that the polarity of solvents ranks among the most significant parameters that influence their efficiency in phenolic compound extraction. For instance, Schieber *et al.* (2003) compared various solvents such as aqueous methanol, acetone, and ethyl acetate in the extraction of phenolic compounds from apple pomace and confirmed that methanol at 80% elicited the greatest total phenolic content (TPC), approximately 2.5 g/kg, as compared to



other solvents. This is because methanol is capable of dissolving phenolic compounds such as chlorogenic acids and various semi-polar phenolic compounds such as quercetin glycosides. This observation was supported by Wijngaard and Brunton (2009), who similarly reported higher yields of hydrophilic antioxidants when polar solvents were used for apple waste extraction, whereas non-polar solvents showed little to no effectiveness in extracting phenolic compounds.

The important role of solvent composition and extraction parameters has also been highlighted through optimization approaches. In a case study by Kahle *et al.* (2005), the influence of alcohol–water mixtures on apple peel extracts was investigated, and optimal results in terms of total phenolic content (TPC; 15 mg/g fresh weight) and DPPH radical scavenging activity above 80% was achieved using 70% ethanol at 40°C for 2 hours. Similarly, Ignat *et al.* (2011) reported that acetone–water mixtures at a 70:30 ratio produced optimal flavonoid extraction from apple peel across different cultivars, with quercetin concentrations ranging from 0.5 to 1.2 mg/g dry weight and corresponding ABTS antioxidant activity.

Importantly, these findings from the studies also highlight the need for the optimal control of, for example, extraction temperatures and durations, as higher temperatures result in the degradation of certain antioxidants, like vitamin C, to a total loss of around 20-30% of their bioactivity.

The environmental and safety concerns that conventional organic solvents pose have been addressed by the emergence of green extraction technologies. Adil *et al.* (2008) pioneered the application of supercritical CO₂ extraction modified with ethanol as a co-solvent for apple seed antioxidants, reaching yields similar to traditional methanol extraction and with minimal toxic residues. More recently, deep eutectic solvents (DES) have shown considerable promise; although specific applications related to *M. domestica* remain limited, studies related to similar fruit matrices suggest that DESs based on choline chloride increase the recovery of polyphenols by 15-25% compared with conventional ethanol extraction while preserving the structural integrity and antioxidant activity of labile compounds.

Other critical issues relate to cultivar-specific responses to solvent extraction. Using methanol extraction, Vrhovsek *et al.* (2004) investigated various apple cultivars and reported that heritage varieties had significantly higher TPC than commercial ones, while the solvent used determined the relative levels of procyanidins to catechins.

This measure of genetic variation in extractability further reinforces the necessity for variety-specific optimization strategies. Even agriculture contributes in this regard, as investigations comparing organic and conventionally cultivated apples have shown that 15-20% greater amounts of flavonoid extract were achieved from organic sources, possibly because of induced synthesis as a stress mechanism resisting pesticides. Assisted extraction techniques have been integrated and are showing better efficiency, with reduced processing time. The UAE with ethanol has been reported to extract 25-30% more antioxidants from apple pomace compared to conventional maceration, while MAE may reach similar values in considerably reduced times. However, these techniques demand precise

control of their parameters to avoid thermal degradation of the bioactive compounds.

With these developments, there are still considerable knowledge gaps. Most of the comparative extraction studies have focused on apple pomace or peel, while few studies have placed attention on flesh tissue. In addition, a head-to-head comparison of aqueous against methanolic extracts, using various complementary antioxidant assays, has seldom been carried out and reported in the literature, especially for a particular cultivar like Red Delicious, grown in tropical and subtropical regions. This study now reports the systematic comparison of aqueous and methanolic extraction of the flesh of Nigerian-grown Red Delicious *M. domestica* using both FRAP and ABTS methodologies.

3. METHODOLOGY

3.1. Material and equipment

Muslin cloth, Micro-pipettes, Spectrophotometer, QASA blender (QBL – 20L330), Glass wares, Analytical balance, Red Apple, Cotton wool, Lyophilizer

3.2. Chemicals and reagents

- *Solvents:* Methanol (80% w/v, prepared from 99.8% analytical grade methanol), Distilled water

- *Assay reagents:* ABTS (≥98%), Potassium persulfate (K₂S₂O₈, ≥99%), DPPH (≥97%), TPTZ (≥97%), Ferric chloride hexahydrate (FeCl₃·6H₂O), Trolox, Ascorbic acid

3.3. Collection and preparation of aqueous and methanolic extracts of *Malus domestica*

3.3.1. Collection and processing of *M. domestica*

Four *M. domestica* fruits (Red Delicious cultivar) were obtained from Shagamu Market, Ogun State, Nigeria. The fruits were peeled and chopped into approximately 1 cm³ pieces. The chopped samples were frozen at –20°C for 24 hours and subsequently lyophilized at –50°C under vacuum (0.1 mbar) for 48 hours using a freeze dryer. The lyophilized samples were then ground into a fine powder using a mechanical grinder and stored in airtight containers at –20°C until extraction.

3.4. Methanolic extraction process of *M. domestica* (Red Delicious cultivar)

For the methanolic extraction of *M. domestica*, 200 g of pulverized sample was macerated in 80% methanol (w/v) at room temperature for 72 hours. The choice of 80% methanol was based on its proven efficiency in extracting a broad spectrum of phenolic compounds, including both polar and semi-polar phytochemicals, while maintaining optimal solubility of flavonoids and hydroxycinnamic acids typically found in apple tissue. The aqueous component (20%) facilitates the extraction of more polar compounds such as anthocyanins and glycosylated flavonoids, providing a more comprehensive phytochemical profile compared to absolute methanol. After maceration, a muslin cloth (0.1 µm pore size) was used to filter the extract, which was concentrated using a rotary evaporator under reduced pressure at 40°C and stored in an air-tight container at 4°C for further procedures. All extractions were performed in triplicate to ensure reproducibility.



3.5. Aqueous extraction process of *M. domestica* (Red Delicious cultivar)

For the preparation of aqueous extracts of *M. domestica*, 200 g of pulverized samples were macerated in 500 mL distilled water at room temperature for 72 hours. After maceration, a muslin cloth (0.1 µm pore size) was used to filter the extracts, which were concentrated using a rotary evaporator under reduced pressure at 40°C and stored in air-tight containers for further procedures.

3.6. ABTS Radical scavenging assay

Free radical scavenging activity of methanolic and aqueous extracts of *M. domestica* was determined by the ABTS radical cation decolorization assay (Christodoulou *et al.*, 2022; Ilyasov *et al.*, 2020). ABTS^{•+} cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12–16 hours before use. ABTS^{•+} solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µL of *M. domestica* extract to 3.995 mL of diluted ABTS^{•+} solution, the absorbance was measured 30 minutes after the initial mixing. An appropriate solvent blank was run in each assay. All measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula:

$$\text{ABTS}^{\bullet+} \text{ scavenging effect (\%)} = [((\text{AB} - \text{AA}) / \text{AB}) \times 100]$$

Where,

AB is the absorbance of ABTS radical + methanol and AA is the absorbance of ABTS radical + sample extract/standard. Trolox was used as the standard substance.

3.7. Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of the *M. domestica* extracts was estimated spectrophotometrically following the procedure of Benzie and Strain (1996), as further described in recent methodological reviews of antioxidant assays (Nwachukwu *et al.*, 2021; Kotha *et al.*, 2022; Christodoulou *et al.*, 2022). The method is based on the reduction of the Fe³⁺-TPTZ complex (colorless complex) to Fe²⁺-tripyridyltriazine (blue colored complex) formed by the action of electron-donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm. The FRAP reagent was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃·6H₂O in the proportion of 10:1:1 at 37 °C. Freshly prepared working FRAP reagent (3.995 mL) was pipetted and mixed with 5 µL of the appropriately diluted *M. domestica* extract and mixed thoroughly. An intense blue color complex was formed when the ferric tripyridyltriazine (Fe³⁺-TPTZ) complex was reduced to the ferrous (Fe²⁺) form, and the absorbance at 593 nm was recorded against a reagent blank (3.995 mL FRAP reagent + 5 µL distilled water) after 30 minutes of incubation at 37°C. All determinations were performed in triplicate. The calibration curve was prepared by plotting the absorbance at 593 nm versus different concentrations of FeSO₄. The concentrations of FeSO₄ were in turn plotted against the concentration of the standard antioxidant Trolox. The FRAP values were obtained by comparing the absorbance change in the test mixture with those obtained from increasing

concentrations of Fe³⁺ and expressed as mg of Trolox equivalent per gram of sample.

4. RESULTS AND DISCUSSION

4.1. FRAP CONTENT OF MEMDF and AEMDF

Table 1. FRAP CONTENT OF MEMDF and AEMDF

FRAP (mg of Trolox equivalent per gram of sample)			
Concentration	MEMDF	AEMDF	Vitamin C
5 µg/mL	BDL	BDL	14.005 ± 0.441
10 µg/mL	1.352 ± 0.156	0.413 ± 0.147	36.355 ± 1.741
25 µg/mL	7.587 ± 0.075	3.968 ± 0.140	51.783 ± 0.399
50 µg/mL	13.886 ± 0.121	10.119 ± 0.279	53.384 ± 0.626
100 µg/mL	25.315 ± 0.149	18.619 ± 0.211	58.844 ± 1.227

KEY

MEMDF: Methanolic Extract of *Malus domestica* Flesh

AEMDF: Aqueous Extract of *Malus domestica* Flesh

BDL: Below Detection Limit

4.2. ABTS CONTENT (mg of Trolox equivalent per gram of sample) of MEMDF and AEMDF

Table 2. ABTS CONTENT (mg of Trolox equivalent per gram of sample) of MEMDF and AEMDF

ABTS (mg of Trolox equivalent per gram of sample)			
Concentration	MEMDF	AEMDF	Vitamin C
5 µg/mL	8.821 ± 0.094	16.334 ± 0.045	49.856 ± 1.729
10 µg/mL	24.222 ± 0.027	18.357 ± 0.417	80.340 ± 0.192
25 µg/mL	31.782 ± 0.300	19.470 ± 0.024	82.271 ± 0.054
50 µg/mL	37.683 ± 4.572	24.964 ± 0.484	82.832 ± 0.675
100 µg/mL	44.923 ± 0.866	27.951 ± 0.907	84.120 ± 0.125

KEY

MEMDF: Methanolic Extract of *Malus domestica* flesh

AEMDF: Aqueous Extract of *Malus domestica* flesh

4.3. Discussion

4.3.1. Ferric Reducing Antioxidant Power (FRAP) of MEMDF and AEMDF

The FRAP assay results presented in Table 1 demonstrate the concentration-dependent antioxidant capacity of both methanolic and aqueous extracts of *M. domestica* flesh. The methanolic extract (MEMDF) exhibited significantly higher ferric reducing power compared to the aqueous extract (AEMDF) across all tested concentrations. At 5 µg/mL, both extracts showed values below detection limit (BDL), indicating minimal antioxidant activity at very low concentrations. However, as the concentration increased to 100 µg/mL, MEMDF displayed a ferric reducing power of 25.315 ± 0.149 mg of Trolox equivalent per gram of sample, while AEMDF showed 18.619 ± 0.211 mg Trolox equivalent/g.

The superior performance of the methanolic extract can be attributed to the enhanced solubility of phenolic compounds



and other antioxidant phytochemicals in organic solvents. Methanol, being a polar organic solvent, is more efficient in extracting a broader spectrum of bioactive compounds, including flavonoids, phenolic acids, and other secondary metabolites that contribute to antioxidant activity. The extraction efficiency differences between polar and non-polar solvents have been well documented in previous studies on plant extracts, where methanolic extractions consistently yielded higher concentrations of polyphenolic compounds.

When compared to the standard antioxidant (ascorbic acid) vitamin C, both extracts demonstrated lower FRAP values. At 100 µg/mL, Vitamin C exhibited a FRAP value of 58.844 ± 1.227 mg Trolox equivalent/g, which is approximately 2.3 times higher than MEMDF and 3.2 times higher than AEMDF. This difference is expected, as Vitamin C is a potent reducing agent with a well-established mechanism of electron donation. Nevertheless, the dose-dependent increase in FRAP values for both *M. domestica* extracts suggests promising antioxidant potential that could be exploited for nutraceutical and pharmaceutical applications.

4.3.2. ABTS radical scavenging activity of MEMDF and AEMDF

The ABTS radical cation decolorization assay (Table 2) allows further investigation into the mechanism of action by which the extracts exert their ability to reduce oxidative stress with an additional method for measuring antioxidant activity. The ABTS results support the FRAP results, as MEMDF shows a substantially greater efficiency in scavenging ABTS; thus, MEMDF had greater antioxidant activity than AEMDF at each concentration evaluated. At the lowest concentration (5 µg/mL), MEMDF had an activity value of 8.821 ± 0.094 mg Trolox equivalent/g, while AEMDF had an activity value of 16.334 ± 0.045 mg Trolox equivalent/g. This trend continued at higher concentrations with MEMDF measuring 44.923 ± 0.866 mg Trolox equivalent/g and AEMDF measuring 27.951 ± 0.907 mg Trolox equivalent/g at 100 µg/mL.

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay allows for the measurement of both hydrophilic and lipophilic antioxidant capacity, making it an appropriate assay to test complex plant extracts. The results obtained with MEMDF confirm our previous hypothesis that methanol extraction yields a greater range of bioactive antioxidant components. The radical scavenging activity of the antioxidants is mediated either by a hydrogen atom transfer (HAT mechanism) or single-electron transfer (SET) from the antioxidant to ABTS^{•+}, leading to the formation of neutralized products and the reduction of reactive oxygen species (ROS).

4.3.3. Concentration-dependent extract performance in ABTS Assay

One interesting observation from the results within the ABTS assay is that, at lower concentration testing, such as 5 µg/mL, the aqueous extract (AEMDF) displayed significantly stronger radical scavenging activity at 16.334 ± 0.045 mg Trolox equivalent/g, while the methanolic extract (MEMDF) exhibited only 8.821 ± 0.094 mg Trolox equivalent/g. This trend, however, reversed when comparing the doses between 10 and 100 µg/mL, in which MEMDF consistently outperformed AEMDF.

Such a concentration-dependent reversal in antioxidant performance could arise from several reasons, considering that the differential extraction and different solubility characteristics of the bioactive compounds are paramount. The aqueous extract, at very low concentrations of 5 µg/mL, may contain higher proportions of readily soluble, highly polar antioxidant compounds, including ascorbic acid (vitamin C), some glycosylated flavonoids, and simple phenolic acids. These water-soluble compounds donate electrons to the ABTS radical cation quickly and efficiently even at minimal concentrations, hence taking immediate effect through radical scavenging activity (Christodoulou *et al.*, 2022).

On the contrary, although the methanolic extract displayed a wide range of polyphenolic constituents such as semi-polar flavonoids, procyanidins, and hydroxycinnamic acid derivatives, it might require a higher concentration to show optimal results in terms of radical scavenging activity. That being said, at a 5 µg/mL concentration, it may not show adequate interaction with ABTS radicals. However, as concentrations vary between 10 and 100 µg/mL, it becomes clear that the antioxidant activity of the diverse components present in the methanolic extract stands out when compared to AEMDF.

Additionally, the ABTS assay measures antioxidant capacity through both hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms (Ilyasov *et al.*, 2020). Water-soluble antioxidants in the aqueous extract may be more efficiently oriented for electron transfer at low concentrations due to their favorable hydration and ionic interactions with the ABTS radical cation in the aqueous-methanolic assay medium. As concentration increases, the superior total phenolic content and structural diversity of compounds in the methanolic extract compensate for any initial kinetic disadvantages, ultimately providing greater overall antioxidant capacity.

This phenomenon underscores the importance of assessing antioxidant activity across multiple concentration ranges and highlights that extraction solvent selection should be guided by the intended application and the specific antioxidant mechanisms of interest.

The reference Vitamin C was found to have more scavenging activity with the ABTS^{•+} assay than any of the extracts with scavenging values of 49.856 ± 1.729 mg Trolox Equivalent with an extract concentration of 5 µg/mL and 84.120 ± 0.125 mg Trolox Equivalent with an extract concentration of 100 µg/mL. Furthermore, the relative difference in activity between the extracts and Vitamin C was less pronounced in the ABTS assay compared to the FRAP assay. As such, it would indicate that *M. domestica* extracts likely use different routes or synergies of antioxidant action which are more efficiently evaluated with the ABTS methodology.

4.3.4. Comparative Analysis of FRAP and ABTS Assays

By evaluating *M. domestica* flesh extracts using both FRAP and ABTS assay simultaneously, we were able to obtain a more extensive assessment of the overall antioxidant profile. Whereas FRAP measures only the reducing ability of the extracts by reducing ferric ions to ferrous ions (reflecting a single ion transfer mechanism), ABTS measures the ability of the extract to quench pre-formed radical cations through many



different mechanisms such as electron transfer or hydrogen atom transfer.

In addition, the fact that MEMDF consistently provides greater antioxidant activity than AEMDF in both assays demonstrates that the extraction solvent used directly affects the potential antioxidant activity of the final extract. Furthermore, the trend toward increased concentration of bioactive compounds being extracted in a dose-dependent manner from both assays indicates that the active components will be present in sufficient quantities to possess antioxidant activity when administered as therapeutics.

5. CONCLUSION

This study presents a scientific comparative analysis of the in vitro study of the antioxidant potential of methanolic and aqueous extracts of *Malus domestica* fruit flesh of Red Delicious cultivar using different assays like FRAP and ABTS. Based on this study, it was found that both methanolic and aqueous extracts found in *M. domestica* have some potential value in protecting the body from oxidative stress. The results of this study proved that the methanolic extract had stronger ferric reducing power at all tested concentrations. Moreover, in ABTS radical scavenging activity tests, it was found that at 5 µg/mL, aqueous extract was more active, and at concentrations between 10 and 100 µg/mL, methanolic extract was more active, achieving 44.923 ± 0.866 mg Trolox equivalent/g at 100 µg/mL. The differential activity of the two extracts in the respective assays at varying concentrations further points to the importance of solvent selection in terms of efficiency as well as the nature of activity in plant-based materials. The studies proposed here, therefore, have significant implications with regard to food science studies. The proposed antioxidant activity of *M. domestica* flesh is relevant in terms of proposing the plant-based materials as food preservatives. In a time when, as a society, consumers are looking for more natural alternatives to synthetic materials, *M. domestica* is a readily acceptable material. In addition, with governmental agencies favoring this type of compound, *M. domestica*-based materials lend themselves well as a means of adding a natural source of antioxidants to foodstuffs.

The information derived from the Nigerian cultivated variety of apples, namely Red Delicious, contributes to the already existing literature on the variation in the content and antioxidant activity of phytochemicals in different cultivars. The information can be used to improve agricultural practices and extraction methods that are cultivar-specific.

Some of the important areas that need further research to transform the findings into practical use are discussed below. First, identification and quantification of specific bioactive compounds that are responsible for the specified antioxidant activities using highly sensitive techniques like HPLC-MS, GC-MS, and NMR are needed to understand the nature of the present phytochemicals. In addition, studies are needed to understand the parameters that could be adjusted to optimize the extraction process and achieve the antioxidant compound extraction in a cost-effective manner. Extending the stability of the extracted antioxidant compounds under different conditions of storage (temperature, light, and pH) and

processing (thermal treatment, changes in pH, and oxidative stress) that are appropriate to food processing practices also need to be evaluated.

Evaluation of the efficacy of these extracts in real-life food systems, including assessing their advantages in preventing lipid oxidation in emulsions, maintaining freshness in cut vegetables and fruits, and compatibility with various food processing operations, would confirm the relevance of these food extracts. Although it is promising that these extracts have good in vitro antioxidant activity, further investigations focusing on biological activities in real-life animal models would be important before determining their relevance for human health-related benefits. Comparing and establishing differences in their antioxidant activities of varying *M. domestica* cultivars and regions would determine better sources for industrial-scale extraction. Additionally, economic analysis and determining the sustainable environment developed through various operations and waste managements would be important.

This study establishes a foundation for the potential utilization of Nigerian-grown Red Delicious apple extracts in food preservation and functional food development, while highlighting the importance of evidence-based extraction strategies for maximizing antioxidant recovery.

REFERENCES

- Adil, I. H., Yener, M. E., & Bayindirli, A. (2008). Extraction of total phenolics of sour cherry pomace by high pressure solvent and subcritical fluid and comparison of the extracts. *Separation Science and Technology*, 43(5), 1091-1110. <https://doi.org/10.1080/01496390701885166>
- Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>
- Bondonno, N. P., Bondonno, C. P., Blekkenhorst, L. C., Considine, M. J., Maghazal, G., Stocker, R., Woodman, R. J., Ward, N. C., Hodgson, J. M., & Croft, K. D. (2018). Flavonoid-rich apple improves endothelial function in individuals at risk for cardiovascular disease: A randomized controlled clinical trial. *Molecular Nutrition & Food Research*, 62(3), 1700674. <https://doi.org/10.1002/mnfr.201700674>
- Carocho, M., Barreiro, M. F., Morales, P., & Ferreira, I. C. F. R. (2014). Adding molecules to food, pros and cons: A review on synthetic and natural food additives. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 377-399. <https://doi.org/10.1111/1541-4337.12065>
- Caruso, G., Torrisi, S. A., Mogavero, M. P., Currenti, W., Castellano, S., Godos, J., Ferri, R., Galvano, F., & Leggio, G.M. (2022). Polyphenols and neuroprotection: Therapeutic implications for cognitive decline. *Pharmacology & Therapeutics*, 232, 108013. <https://doi.org/10.1016/j.pharmthera.2021.108013>
- Christodoulou, M. C., Orellana Palacios, J. C., Hesami, G., Jafarzadeh, S., Lorenzo, J. M., Domínguez, R., Moreno, A., & Hadidi, M. (2022). Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals. *Antioxidants*, 11(11), 2213. <https://doi.org/10.3390/antiox11112213>



- Dehghani Zahedani, A., Shariat Torbaghan, S., Rahili, S., Karlin, K., Scilley, D., Thakkar, R., Saberi, M., Hashemi, N., Perelman, D., Aghaeepour, N., McLaughlin, T., & Snyder, M. P. (2021). Improvement in glucose regulation using a digital tracker and continuous glucose monitoring in healthy adults and those with type 2 diabetes. *Diabetes Therapy*, 12(7), 1871-1886. <https://doi.org/10.1007/s13300-021-01092-x>
- Durazzo, A., & Lucarini, M. (2019). Extractable and non-extractable antioxidants. *Molecules*, 24(10), 1933. <https://doi.org/10.3390/molecules24101933>
- Forman, H. J., Zhang, H., & Rinna, A. (2009). Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Molecular Aspects of Medicine*, 30(1-2), 1-12. <https://doi.org/10.1016/j.mam.2008.08.006>
- Geană, E.-I., Ciucure, C. T., Ionete, R. E., Ciocârlan, A., Aricu, A., Fica, A., & Andronescu, E. (2021). Profiling of phenolic compounds and triterpene acids of twelve apple (*Malus domestica* Borkh.) cultivars. *Foods*, 10(2), 267. <https://doi.org/10.3390/foods10020267>
- Grabska-Kobyłecka, I., Szpakowski, P., Król, A., Książek-Winiarek, D., Kobyłecki, A., Głabiński, A., & Nowak, D. (2023). Polyphenols and their impact on the prevention of neurodegenerative diseases and development. *Nutrients*, 15(15), 3454. <https://doi.org/10.3390/nu15153454>
- Graziani, G., Gaspari, A., Di Vaio, C., Cirillo, A., Ronca, C. L., Grosso, M., & Ritieni, A. (2021). Assessment of in vitro bioaccessibility of polyphenols from Annurca, Limoncella, Red Delicious, and Golden Delicious apples using a sequential enzymatic digestion model. *Antioxidants*, 10(4), 541. <https://doi.org/10.3390/antiox10040541>
- Gülçin, İ. (2020). Antioxidants and antioxidant methods: An updated overview. *Archives of Toxicology*, 94(3), 651-715. <https://doi.org/10.1007/s00204-020-02689-3>
- Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food Chemistry*, 126(4), 1821-1835. <https://doi.org/10.1016/j.foodchem.2010.12.026>
- Ilyasov, I. R., Beloborodov, V. L., Selivanova, I. A., & Terekhov, R. P. (2020). ABTS/PP decolorization assay of antioxidant capacity reaction pathways. *International Journal of Molecular Sciences*, 21(3), 1131. <https://doi.org/10.3390/ijms21031131>
- Kotha, R. R., Tareq, F. S., Yildiz, E., & Luthria, D. L. (2022). Oxidative stress and antioxidants—A critical review on in vitro antioxidant assays. *Antioxidants*, 11(12), 2388. <https://doi.org/10.3390/antiox11122388>
- Koutsos, A., Tuohy, K. M., & Lovegrove, J. A. (2015). Apples and cardiovascular health—Is the gut microbiota a core consideration? *Nutrients*, 7(6), 3959-3998. <https://doi.org/10.3390/nu7063959>
- Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, 13, 757-772. <https://doi.org/10.2147/CIA.S158513>
- Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, 24(22), 4132. <https://doi.org/10.3390/molecules24224132>
- Nwachukwu, I. D., Sarteshnizi, R. A., Udenigwe, C. C., & Aluko, R. E. (2021). A concise review of current in vitro chemical and cell-based antioxidant assay methods. *Molecules*, 26(16), 4865. <https://doi.org/10.3390/molecules26164865>
- Parmenter, B. H., Croft, K. D., Hodgson, J. M., Dalgaard, F., Bondonno, C. P., Lewis, J. R., Cassidy, A., Scalbert, A., & Bondonno, N. P. (2020). An overview and update on the epidemiology of flavonoid intake and cardiovascular disease risk. *Food & Function*, 11(8), 6777-6806. <https://doi.org/10.1039/D0FO01118E>
- Schieber, A., Hilt, P., Streker, P., Endreß, H. U., Rentschler, C., & Carle, R. (2003). A new process for the combined recovery of pectin and phenolic compounds from apple pomace. *Innovative Food Science & Emerging Technologies*, 4(1), 99-107. [https://doi.org/10.1016/S1466-8564\(02\)00087-5](https://doi.org/10.1016/S1466-8564(02)00087-5)
- Shahidi, F., & Peng, H. (2025). Dietary phenolics and their antioxidant activities. In R. Watson, V. Preedy, & S. Zibadi (Eds.), *Polyphenols: Mechanisms of action in human health and disease* (3rd ed., pp. 105-118). Academic Press. <https://doi.org/10.1016/B978-0-443-21591-9.00009-3>
- Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology*, 21(7), 363-383. <https://doi.org/10.1038/s41580-020-0230-3>
- Sies, H., Belousov, V. V., Chandel, N. S., Davies, M. J., Jones, D. P., Mann, G. E., Murphy, M. P., Yamamoto, M., & Winterbourn, C. (2022). Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nature Reviews Molecular Cell Biology*, 23(7), 499-515. <https://doi.org/10.1038/s41580-022-00456-z>
- Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual Review of Biochemistry*, 86, 715-748. <https://doi.org/10.1146/annurev-biochem-061516-045037>
- Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry*, 52(21), 6532-6538. <https://doi.org/10.1021/jf049317z>
- Wijngaard, H. H., & Brunton, N. (2009). The optimization of extraction of antioxidants from apple pomace by pressurized liquids. *Journal of Agricultural and Food Chemistry*, 57(22), 10625-10631. <https://doi.org/10.1021/jf902498y>

