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Investigation of antioxidant properties in the aqueous extract of apple (*Malus pumila*)

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Abstract

This research examined the antioxidant properties of *Malus pumila* (Granny Smith apples) extract using a range of in vitro assays. The antioxidant potential was assessed through five methods: DPPH (2,2-diphenyl-1-picryhydrazyl radical) and ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical scavenging activities, ferric reducing antioxidant power (FRAP), hydrogen peroxide scavenging, and measurement of ascorbic acid content. Results showed that the extract exhibited notable antioxidant activity, especially at lower concentrations (10–20 µl/ml), where it performed comparably to, and in some cases better than, the standard antioxidant, ascorbic acid. In both the DPPH and ABTS assays, the extract showed strong free radical scavenging capabilities, suggesting its effectiveness in protecting cells from oxidative damage. The extract also demonstrated a good ability to reduce ferric ions (Fe^{3+}) to their more bioavailable ferrous form (Fe^{2+}), reinforcing its ability to improve antioxidant defense systems. Although its hydrogen peroxide scavenging capacity was lower than that of ascorbic acid, the overall performance of *Malus pumila* suggests it is a valuable natural source of antioxidants. These results show the fruit's potential use in managing oxidative stress-related conditions and support its possible application in health-promoting diets or therapeutic formulations.

Keywords: *Malus pumila*; Antioxidants; Ascorbic Acid; Hydrogen peroxide

1. Introduction

Apples (*Malus pumila*) are among the most extensively consumed foods in the world. In addition to fresh eating, apples are widely processed into a range of commercial goods, the most well-known of which being apple juice. However, the juicing procedure dramatically reduces the nutritional and functional value of the fruit, particularly in terms of total phenolic content (TPC) and antioxidant capacity. Apple juice made using traditional pressing processes can lose up to 58% of its phenolic components [1]. Furthermore, Van Der Sluis *et., al* [2] found that juicing loses up to 90% of antioxidant activity, as well as losses in essential bioactive chemicals including chlorogenic acid (by 50%) and catechins (by 3%). This significant loss of phenolics and antioxidants is attributed to their retention in apple pomace, a fibrous byproduct of juice extraction that contains a big part of the fruit's bioactive constituents [2,3,4]. Apples are notable for their high content of bioactive chemicals. Pectins, dietary fibers, oligosaccharides, triterpenic acids, and a variety of phenolic compounds, including flavonols, dihydrochalcones, anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids, are examples. Apples are well-known for their high total phenolic content, which frequently exceeds 20 mmol Trolox equivalents per kilogram and plays a crucial role to antioxidant activity [5-8]. The Food and Agriculture Organization (FAO) estimates that global apple production will top 86 million metric tons in 2020. From 2012 to 2020, Asia has the biggest production share (64.1%), followed by Europe (20%) and the Americas (11.6%). Poland, France, and Italy produced more than 7.6 million tons of apples in 2020. By 2022, global production had increased to around 95.84 million metric tons. However, USDA predictions for 2024/25 expect a decrease to 84 million tons, owing to lower yields in the European Union, the United States, Turkey, and Russia. In terms of consumption, the

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United States had the highest per capita intake in 2021, at 51.9 kg, with Hungary and Turkey following closely. In 2022, the average person in the United States consumed about 15.77 pounds (7.15 kg) of fresh apples. Despite sparse global consumption data, these estimates indicate that apple consumption remains important, particularly in high-producing and health-conscious countries. The full apple genome was sequenced in 2010, which was a big step in figuring out its phytochemical and genetic composition [9]. Apples are naturally low in fat, salt, and cholesterol, and high in dietary fiber (particularly pectin), vitamin C, vitamin B-complex (e.g., B6, riboflavin, thiamin), and antioxidants (e.g., quercetin, catechin, chlorogenic acid). Conceição de Oliveira *et., al* [10] discovered that overweight women who consumed three apples or pears daily while on a calorie-restricted diet lost more weight than those who did not. Harvard researchers reported that eating flavonoid-rich foods like apples on a daily basis may help prevent weight gain [11]. Granny Smith apples, in particular, have high levels of nondigestible chemicals that promote healthy gut flora, hence lowering obesity-related metabolic diseases [12]. Apples contain chemicals with anti-cancer properties. Liu R.H., Liu, J., and Chen, B. [13] found dose-dependent decreases in breast tumor formation in animal models. Apples are high in quercetin, which has been linked to a lower incidence of pancreatic and prostate cancer [14, 15]. Apple peel extracts have been shown to prevent the multiplication of cancer cells in the colon, breast, and liver [16]. Daily apple consumption has also been linked to lower colorectal cancer risk [17]. Numerous research support apples' cardioprotective properties. Apple eating was linked to lower coronary heart disease mortality in postmenopausal women [18]. A French cohort study confirmed apples' positive effects on cardiovascular risk variables such as blood pressure and cholesterol levels [19]. Ranked among the top 20 antioxidant-rich foods by the USDA, apples obtain nearly two-thirds of their antioxidant content from the peel [20]. These antioxidants scavenge free radicals and attenuate oxidative stress, aiding disease prevention. Because of their ability to reduce oxidative stress, antioxidants are becoming more and more acknowledged as necessary food additives and nutraceutical supplements. Scientific papers on antioxidants and their uses in a variety of industries have significantly increased over the last ten years [21–25]. Antioxidants' main function in food systems is to suppress oxidative processes by either stopping the production of free radicals or blocking the routes that allow them to spread [26–28]. Reactive oxygen species (ROS) and other free radicals are neutralized by antioxidants, which are bioactive substances that prevent oxidative damage and shield cellular constituents from oxidative stress [29]. By giving free radicals electrons, they act as reducing agents, stopping chain reactions that harm proteins, lipids, and nucleic acids. Based on their source, function, and solubility, antioxidants can be widely grouped. While lipid-soluble antioxidants, such as α-tocopherol, carotenoids, and coenzyme Q10, are mainly found in cell membranes, where they prevent lipid peroxidation and preserve membrane integrity, water-soluble antioxidants, such as ascorbic acid, glutathione, and lipoic acid, function in aqueous environments like blood plasma and the cytosol [30]. Antioxidants are divided into nutritional, enzymatic, and synthetic categories based on their sources. Vitamins A, C, and E, carotenoids (including β-carotene and lycopene), and trace minerals (such zinc and selenium) are examples of nutrient antioxidants that are acquired from diet. ROS are neutralized by endogenous enzymatic antioxidants such as glutathione peroxidase, catalase, and superoxide dismutase (SOD) through catalytic processes. Furthermore, by sequestering transition metals, metal-binding proteins like ferritin and ceruloplasmin inhibit the production of ROS. Natural alternatives like flavonoids and tocopherols are becoming more popular as a result of concerns about the long-term safety of synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are widely used in industrial applications [31, 32]. Fruits such as *Malus pumila* (apple) are rich in bioactive chemicals that impart significant health advantages, frequently surpassing synthetic antioxidants in terms of bioavailability and safety. However, the antioxidant potential of apples remains underrecognized, particularly in ordinary dietary choices. This study uses a variety of in vitro spectrophotometric assays, such as DPPH, ABTS, FRAP, hydrogen peroxide scavenging, and ascorbic acid tests, to assess the antioxidant activity of aqueous apple extract. The research seeks to demonstrate the therapeutic value of apples as a natural antioxidant source by contrasting their effectiveness with that of regular ascorbic acid. This will promote the use of apples in preventative and functional nutrition strategies.

2. Antioxidant Compounds Present in Apples

Apples have gained considerable attention due to their rich chemical composition, particularly their potent antioxidant profile. Among the natural antioxidants present, phenolic compounds are recognized as the principal contributors to the fruit's antioxidant properties. Additionally, apples serve as an excellent source of antioxidant vitamins, especially vitamins C and E. Vitamin C, predominantly found in ascorbic acid and its oxidized form (dehydroascorbic acid), is present at concentrations ranging from 2 to 35 mg/100 g depending on the apple variety, placing apples as the second richest fruit in vitamin C after cranberries. This vitamin exhibits notable radical-scavenging activity (EC_{50} = 0.35). Vitamin E, primarily located in the seeds and apple pomace, also shows significant antioxidant potential (EC_{50} = 0.30), with a reported concentration of 5.5 mg/100 g. Although present only in trace amounts, vitamins B_{12} and D also contribute minimally to the fruit's overall antioxidant potential. Phenolic compounds in apples comprise over 60 identified structures, broadly categorized into phenolic acids and flavonoids. Phenolic acids include both hydroxybenzoic (e.g., gallic acid, protocatechuic acid, vanillic acid, syringic acid) and hydroxycinnamic acids (e.g., caffeic, chlorogenic, p-coumaric acids), most of which are found in conjugated forms and are more concentrated in the apple

peel than in the flesh. Flavonoids, another major class of polyphenols in apples, are distributed across four main subclasses: flavonols (71–90%), flavan-3-ols (1–11%), anthocyanins (1–3%), and chalcones/dihydrochalcones (2–6%). These compounds are structurally characterized by one or more hydroxylated aromatic rings, enabling them to participate in redox reactions that neutralize free radicals and confer multiple health benefits.

3. Materials and methods

3.1. Sample Collection and Preparation of Extract

Fresh green apples (*Malus pumila*) were obtained in May from Tipper Garage Market, located in the Tanke area of Ilorin, Kwara State, Nigeria. Following collection, the samples were thoroughly cleaned with distilled water to remove any surface contamination. The cleaned apples were then cut and homogenized in a laboratory blender to create a homogeneous pulp. The resultant homogenate was passed through a fine-mesh screen to separate the juice (aqueous extract) from the solid leftovers. The clear extract was then collected and refrigerated until further study.

Figure 1 Extraction of aqueous apple juice using a sieve after homogenization

3.2. Reagents and Chemicals

All reagents used in this study were analytical grade. The substances listed below were purchased from Santa Cruz Biotechnology, Dallas, Texas, USA: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Horse Radish Peroxidase (HRP), 3,5-dichlorohydroxybenzene sulfonic acid, 4-aminoantipyrine, and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ). Loba Chemie of India supplied ethanol, disodium monophosphate, potassium dihydrogen phosphate, potassium persulfate, ferric chloride, and ferrous sulfate. All reagents have been preserved according to the manufacturer's instructions and freshly produced for each test.

3.3. Assessment of Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity

The free radical scavenging ability of the aqueous extract of *Malus pumila* was assessed using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay as described by [33], with slight modifications. Using a slightly modified version of [33] DPPH (2,2-Diphenyl-1-1-picrylhydrazyl) test, the aqueous extract of *Malus pumila* was evaluated for its capacity to scavenge free radicals. Briefly, various amounts of the extract and standard (ascorbic acid) in phosphate buffer were made (10, 20, 50, 100, 150, and 200 µg/ml). After transferring a 20 µL aliquot of each solution into the microplate's wells, 160 µL of phosphate buffer (pH 7.2) was added. 40 µL of 0.2 mM DPPH in ethanol were then added. A microplate reader was used to measure absorbance at 517 nm after the mixture was incubated for 15 minutes at room temperature. The radical scavenging activity was calculated using the formula:

$$DPPH\ scavenging\ activity(\%) = A_0 - A_1/A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample

3.3.2. ABTS Radical Scavenging Activity

The ABTS radical scavenging activity was determined using the method of [34] with modifications. The ABTS radical cation (ABTS•⁺) was produced by reacting 5 mL of 14 mM ABTS solution with 5 µL/mL of 4.9 mM potassium persulfate ($K_2S_2O_8$), followed by storage in the dark at room temperature for 16 hours. Prior to use, the solution was diluted with ethanol to achieve an absorbance of 0.700 ± 0.020 at 734 nm. For the assay, 20 µL of the sample or standard (ascorbic acid) at concentrations of 10, 20, 50, 100, 150, and 200 µg/ml was added to each well along with 160 µL phosphate buffer (pH 7.2), followed by 40 µL of the ABTS•⁺ solution. After a 15-minute incubation at room temperature, absorbance was measured at 734 nm. The scavenging activity was calculated using the formula:

$$ABTS\ scavenging\ activity(\%) = A_0 - A_1/A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample or standard

3.3.3. Hydrogen Peroxide (H_2O_2) Scavenging Activity

The H_2O_2 scavenging activity of the extract was assessed using a modified method of [35]. A 43 mM hydrogen peroxide solution was prepared in 0.1 M phosphate buffer (pH 7.4). Sample and standard (ascorbic acid) solutions (50 µL) at concentrations of 10, 20, 50, 100, and 150 µg/ml were combined with 100 µL phosphate buffer and 50 µL of the H_2O_2 solution in microtiter wells. After a 15-minute incubation, 600 µL of phosphate buffer was added to each well to bring the final volume to 1 mL. Subsequently, 300 µL of chromogenic solution (containing 0.25 mM 4-aminoantipyrine, 2 mM 3,5-dichloro-2-hydroxybenzenesulfonic acid, and 0.5 U/L HRP in 150 mM potassium phosphate buffer, pH 7.0) was added. The formation of the red quinoneimine dye was monitored by measuring absorbance at 520 nm. The scavenging activity was calculated using the formula:

$$H_2O_2\ scavenging\ activity(\%) = A_0 - A_1/A_0 \times 100$$

Where A_0 is the absorbance of the blank and A_1 is the absorbance of the sample

3.3.4. Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing ability of the apple extract was determined using the FRAP assay method developed by [35], with modifications. The FRAP reagent was freshly prepared by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1 (v/v/v). A 20 µL aliquot of the extract or standard at various concentrations (10, 20, 50, 100, and 150 µg/ml) was added to 300 µL of the FRAP reagent. The reaction mixture was incubated at 37 °C for 30 minutes, and the absorbance was measured at 593 nm. The antioxidant potential was quantified using a calibration curve of ferrous sulfate and expressed in mmol $FeSO_4$ equivalents per gram dry weight (DW) of the extract.

3.3.5. Ascorbic Acid Assay

The ascorbic acid content of the aqueous extract was determined based on the method described by [37], with slight modifications.

- **Principle:** Phosphomolybdate is stoichiometrically reduced by ascorbic acid in the presence of inorganic phosphorus to form a blue-colored molybdenum complex, which exhibits maximum absorbance at 660 nm.
- **Reagents:** All reagents were prepared using distilled water. These include: Sodium molybdate (3.33%), Sulfuric acid (H_2SO_4, 0.25 N), Disodium monophosphate (0.25 mM), Trichloroacetic acid (TCA, 5% w/v)

Procedure: A volume of 20 µL of 5% TCA was added to 1 mL of the apple extract sample. The mixture was centrifuged at 3500 rpm for 10 minutes at 4 °C to obtain the clear supernatant. Subsequently, 0.5 mL of the supernatant was combined with 0.2 mL sodium molybdate (0.66%), 0.2 mL of 0.05 N sulfuric acid, and 0.1 mL of 0.025 mM disodium phosphate. The mixture was incubated in a water bath at 60 °C for 40 minutes. After incubation, the reaction mixture was cooled under running water and centrifuged at 4000 rpm for 5 minutes. The absorbance of the resulting clear supernatant was measured at 660 nm using a spectrophotometer against an appropriate reagent blank. Ascorbic acid standards prepared in microgram concentrations were used to construct a standard curve. The ascorbic acid content in the sample was extrapolated from the calibration curve and expressed as µg ascorbic acid per mL of sample.

The following equation was used to compute the ascorbic acid concentration:

$$Ascorbic\ acid\ content(\mu g/ml) = (A_1 - 0.0549) \times 100$$

Where A_1 = Absorbance of the sample

3.4. Statistical Analysis

All quantitative data obtained from antioxidant assays were expressed as mean ± standard error of mean (SEM). Statistical significance among treatment groups was evaluated using one-way analysis of variance (ANOVA). Post hoc analysis was performed using Duncan's Multiple Range Test at a confidence level of 95% ($p < 0.05$) to determine significant differences among group means. All statistical analyses were carried out using appropriate statistical software.

4. Results and Discussion

4.1. Ascorbic acid assay

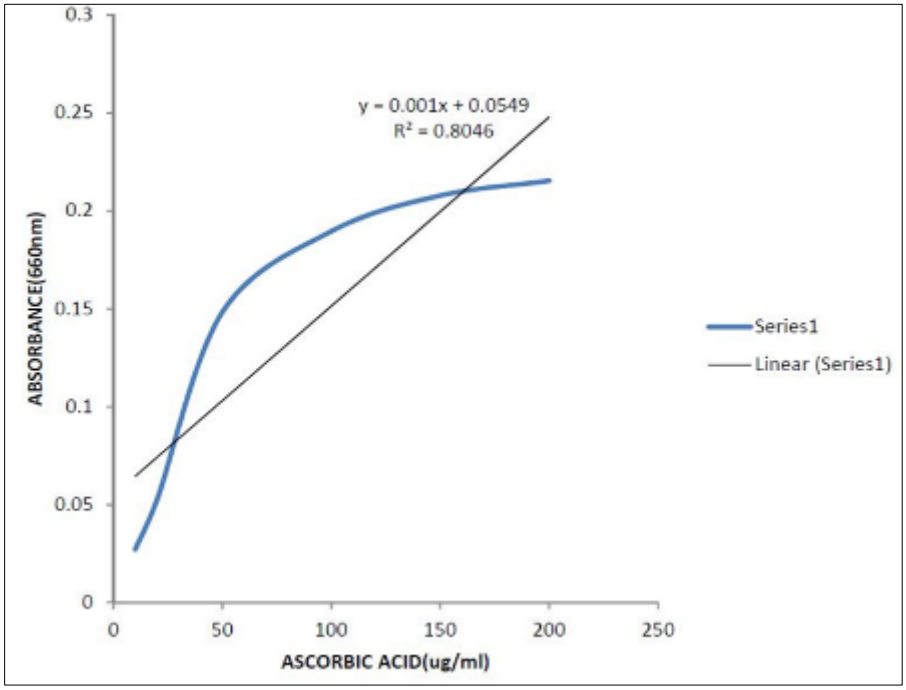

Figure 2 Ascorbic Acid Assay chart

Ascorbic Acid analysis for *Malus pumila* (apple) extract was performed with a spectrophotometric technique for absorbance at 660 nm. Once absorbance was recorded, a standard calibration curve was generated to relate absorbance to the concentration of ascorbic acid, with concentrations ranging from 0–250 µg/mL of ascorbic acid. The concentration of ascorbic acid in *Malus pumila* extract was 806 µg/mL, which exceeded the upper range of the standard curve of 250 µg/mL. This indicates that the sample likely required dilution before being analyzed. The example of high ascorbic acid content in *Malus pumila* (apple) indicates that the fruit could be considered one source of the essential vitamin, in line with our goal of finding sources of vitamins. Fresh extracts were analyzed for ascorbic acid content in accordance with the work of [38], who concluded that the ascorbic acid content will degrade over time in stored samples. Although the assay represents a practical means to understand ascorbic acid concentrations in *Malus pumila* sample extracts, the moderate R^2 and extrapolation beyond the standard range shows that improvements can and will be made. The reported high concentration from *Malus pumila* further indicates potential as a vitamin C source, in keeping with research suggesting that fruit be eaten fresh for maximum nutrient retention.

4.2. ABTS Radical Scavenging Activity

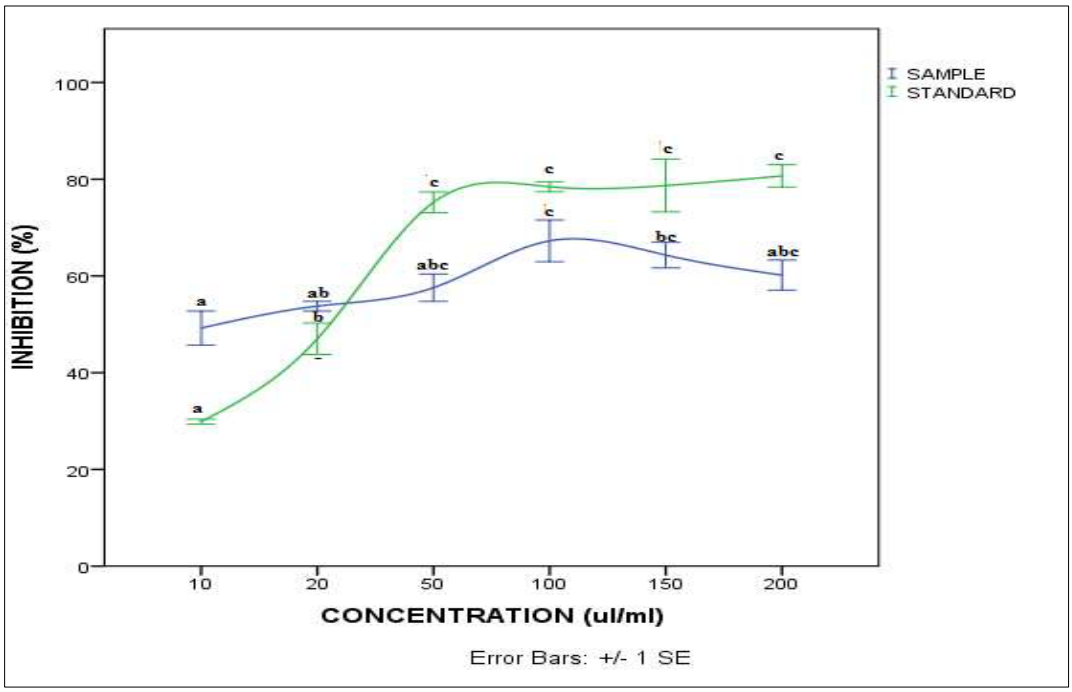

Figure 3 ABTS Radical Scavenging Activity chart

Figure 3 illustrates the ABTS radical scavenging activity of *Malus pumila* aqueous extract compared to ascorbic acid (standard) across concentrations ranging from 10 to 200 µL/mL. The inhibition profile of both the sample and standard was concentration-dependent, with both showing progressive increases in scavenging activity. Interestingly, at the lowest concentration tested (10 µL/mL), the extract exhibited exceptional radical inhibition (49.23%) compared to the standard (29.88%). A similar trend was observed at 20 µL/mL, where the extract exhibited 53.76% inhibition, outperforming the standard (47.01%). These observations indicate that *Malus pumila* extract is more effective at scavenging ABTS radicals than ascorbic acid at lower concentrations, indicating the existence of potent antioxidant constituents within the extract that act efficiently at minimal dosages. As the concentration increased, the standard antioxidant exhibited a more rapid and sustained increase in inhibition, peaking around 80–85% from 50 µL/mL onward and remaining stable across higher concentrations. In contrast, the extract peaked at 100 µL/mL (approximately 70% inhibition) but demonstrated a slight decline in scavenging activity at 150 µL/mL and 200 µL/mL, although the differences were not statistically significant from the standard at those concentrations. The observed decline at higher doses could be attributed to the saturation or antagonistic interactions of phytochemical constituents at elevated concentrations. Overall, the *Malus pumila* extract showed appreciable ABTS radical scavenging activity, particularly at lower concentrations, emphasizing its potential as a natural source of antioxidants for therapeutic and nutritional applications.

4.3. DPPH radical scavenging activity

Figure 4 illustrates the DPPH radical scavenging activity of *Malus pumila* aqueous extract compared to ascorbic acid across a concentration range of 10–200 µL/mL. The graph reveals a general concentration-dependent increase in radical scavenging activity for both the sample and standard. Notably, at the lowest concentration of 10 µL/mL, the extract exhibited a scavenging activity of 50.14%, which was slightly higher than that of ascorbic acid (49.56%). This finding shows the extract's capacity to neutralize DPPH radicals effectively at low concentrations. Across all tested concentrations, the numbers showed no big differences between the inhibitory activities of the extract and the standard, suggesting comparable antioxidant potential. The extract maintained a relatively moderate level of DPPH scavenging activity across the concentration range, with values plateauing between 53–59%. While the standard (ascorbic acid) showed a more pronounced increase in scavenging activity—reaching a peak of approximately 80% at higher concentrations—the extract displayed a stable but limited increase in efficacy. This trend suggests that although *Malus pumila* extract does not match the high-concentration efficiency of ascorbic acid, it exhibits a reliable antioxidant response, particularly at low concentrations. The similar effect of the extract at lower doses demonstrates its capability to protect biological systems from oxidative stress as a result of the presence of biologically active constituents such as polyphenols and flavonoids.

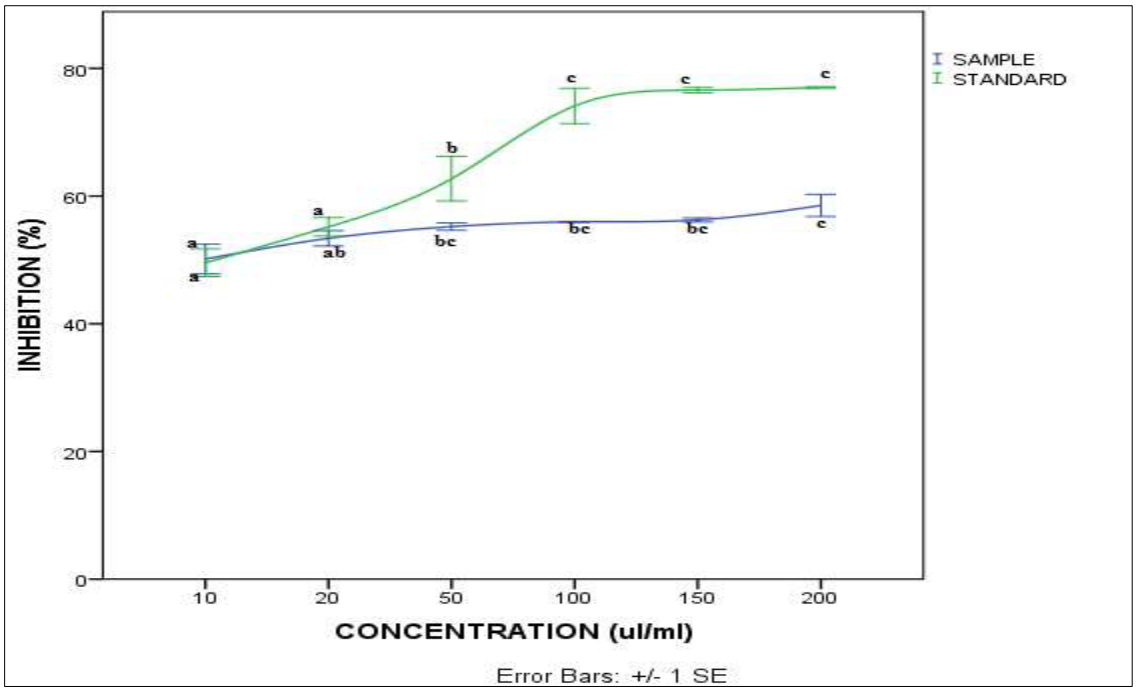

Figure 4 DPPH Radical Scavenging Activity chart

4.4. Ferric Reducing Antioxidant Power (FRAP) Assay

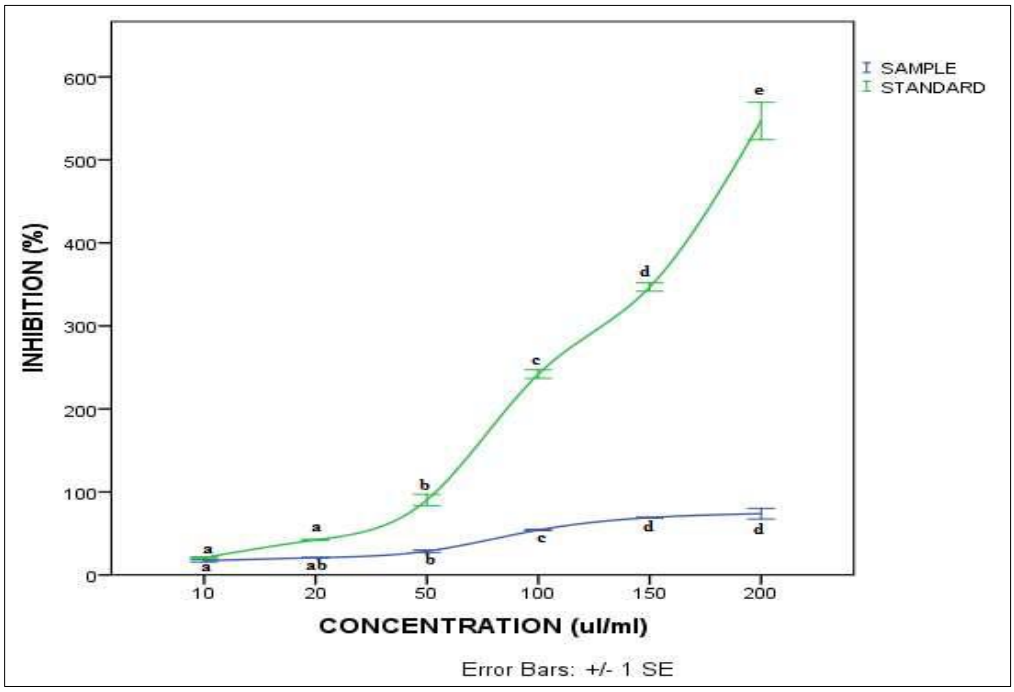

Figure 5 Ferric Reducing Antioxidant Power Chart

Figure 5 shows the ferric reducing antioxidant power (FRAP) of *Malus pumila* aqueous extract compared to ascorbic acid over a concentration range of 10–200 µL/mL. The data indicates that the reducing power of both the sample and standard progressively increased with concentration, confirming a concentration-dependent antioxidant response. At the lowest concentration included in the study (10 µL/mL), *Malus pumila* extract showed a significant ability to convert ferric (Fe^{3+}) into ferrous ions (Fe^{2+}). Benchmarked against ascorbic acid at this concentration, the inhibition of ferric ions was not significantly different over all of the tested values. This becomes an interesting preliminary finding given that it is fairly established that the *Malus pumila* extract possesses biologically relevant antioxidant constituents. That

said, while both the extract and standard were similarly proficient at reducing ferric ions over the lower concentrations (10–150 µL/mL), they diverged substantially at 200 µL/mL, with the standard showing demonstrably superior activity. While the extract continued to show increases in ability to redox ferric ions, it always remained lower than the ascorbic acid standard as concentration rose. Importantly, at 200 µL/mL, the ascorbic acid standard showed a ferric ion redox percentage of over 500%, indicated a substantially better performance compared to the extract. Given the observations of ascorbic acid, its implication of its general performance stationary, the significant escalation in reducing power seems to indicate a higher propensity to donate electrons, an important skill to help neutralize oxidative species. Nonetheless, the effectiveness of *Malus pumila* extract in reducing Fe^{3+} at lower concentrations supports its possible role in alleviating oxidative stress through redox homeostasis. This finding is of relevance in terms of nutraceutical development in circumstances that call for moderate antioxidant support and not the vigorously high activities of synthetic antioxidants.

4.5. Hydrogen Peroxide Radical Scavenging Activity

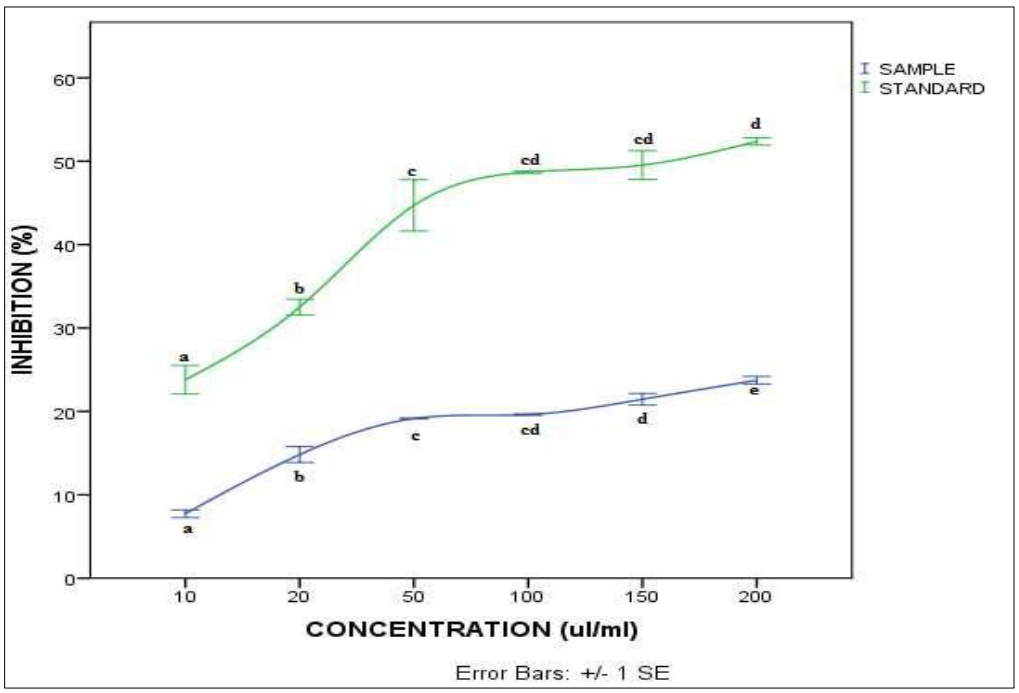

Figure 6 Hydrogen Peroxide Radical Scavenging Activity

Hydrogen peroxide is a cytotoxic agent whose levels must be minimized and *Malus pumila* extract has shown the ability to scavenge hydrogen peroxides at higher concentrations, albeit lower than the standard at same concentration. The extract did not present a good ability to scavenge hydrogen peroxide radicals as efficiently as the ascorbic acid standard used. *Malus pumila* extract exerted a concentration-dependent scavenging of hydrogen ascorbic acid with an activity of 52.38% at the same concentration of 200µl/ml. There was no statistical difference between the sample and standard at concentrations of 10µl/ml, 20µl/ml, 50µl/ml and 100µl/ml.

5. Conclusion

The results of this research demonstrate that *Malus pumila* possesses appreciable antioxidant properties, particularly at lower concentrations. The extract exhibited moderate but significant free radical scavenging abilities, as seen in the ABTS and DPPH assays, and a capacity for ferric ion reduction in the FRAP assay. Notably, its performance was comparable to that of ascorbic acid at lower concentrations, showing its ability as a natural antioxidant source. The concentration-dependent activity observed suggests that the extract contains bioactive compounds capable of neutralizing oxidative stress, a major contributor to cellular damage and various chronic diseases.

Collectively, these findings show the therapeutic promise of *Malus pumila* in managing conditions linked to oxidative damage, including those involving abnormal cell proliferation or tumor growth. Its effectiveness at low concentrations further supports its use in preventive healthcare and natural antioxidant-based therapies. Therefore, *Malus pumila*

could function as an essential component in the development of nutraceuticals or functional foods aimed at combating oxidative stress and enhancing cellular health.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares no competing interests. This manuscript has not been submitted to, nor is under review at another journal or publishing site.

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