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SHORT COMMUNICATION

In Vitro Studies on Antioxidant Potential of Apple (*Malus domestica*) Fructus Extract Nanoparticle

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Abstract

Oxidative stress results from an imbalance of free radicals and antioxidants in the body. Antioxidants are needed to prevent oxidative stress. A diet rich in fruits and vegetables, which are high in antioxidants, should help avoid oxidative stress. One source of antioxidants is apples (*Malus domestica*) from the Rosaceae family because they have some bioactive compounds such as catechin, chlorogenic acid, quercetin, and phloridzin. Recently, many studies have used nanotechnology to formulate plant extracts. Due to their size and distinctive physicochemical properties, nanoparticles in plant extracts have various benefits. Analyzing apple extract nanoparticles' antioxidant capacity was the goal of this work. The synthesized nanoparticles of apples were made by using chitosan, glacial acetic acid, propylene glycol, ethanol, DMSO, and Na-TPP. A dynamic light scattering particle size analyser was used to measure the zeta potential and particle size. Antioxidant activity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, Hydrogen Peroxide (H_2O_2) scavenging activities, and Ferric Reducing Antioxidant Power (FRAP) assay using colorimetric methods. At a concentration of 100 g/ml, the most DPPH was scavenged (80.35%). Apple extract nanoparticles have strong DPPH scavenging activity with IC_{50} = 12.16 ± 2.98 µg/ml and H_2O_2 scavenging activity with IC_{50} = 81.96 ± 7.23 µg/ml. The highest H_2O_2 scavenging activity was at 200 µg/ml concentration (84.47%) and the highest FRAP activity was at a concentration of 50 µg/ml (444.29%). The concentration is directly proportional to the antioxidant activity of apple extract nanoparticles. Based on this study, apple extract nanoparticle has strong antioxidant activity.

Keywords: Antioxidant, DPPH, FRAP, H_2O_2, *Malus domestica,* Nanotechnology

1. Introduction

Reactive substances known as free radicals have an unpaired electron in their outer orbit. Reactive Oxygen Species (ROS) include free radicals as well as oxygen-containing molecules that are not free radicals, including molecules like Hydrogen Peroxide (H_2O_2), Superoxide (O_2), Singlet Oxygen ($1/2O_2$), and the Hydroxyl radical (OH). When our cells use food and oxygen to produce energy, as well as when we are exposed to microbe infections, vigorous exercise, or pollutants/toxins like cigarette smoke, alcohol, ionizing and UV radiation, pesticides, and ozone, free radicals are created. An excess of ROS that emerges from an imbalance between the creation and clearance of ROS characterizes oxidative stress (the latter is regulated by antioxidant defences). The endogenous antioxidant defence may benefit from a greater intake of exogenous antioxidants from fruits and vegetables because oxidative damage to our cells gets worse with age. Currently, the main exogenous antioxidants are thought to be antioxidants like vitamins C and E, carotenoids,

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and polyphenols (like flavonoids). According to clinical studies, individuals can avoid disease by consuming a diet high in vegetables, fruits, legumes, whole grains, and omega-3 fatty acids[1].

The discovery of diverse products for medicine development can be found in plants. Several compounds derived from plants are now widely used medications in several nations across the world[2]. Numerous epidemiological and biochemical research conducted over the past few decades have demonstrated the positive benefits that fruits and vegetables have on human health. There is significant evidence that eating fruits and vegetables, which are abundant in vitamins and fiber, lowers the risk of degenerative diseases like cancer, cardiovascular disease, and cataracts[3]. This relates to the anti-oxidants found in fruits and vegetables, such as vitamins C and E, carotenoids, and polyphenols, which shield the body from free radical damage[4].

A diet rich in fruits and vegetable should help minimize oxidative stress because these foods are high in antioxidants. This should delay aging and help fight chronic diseases. Many commonly consumed foods and beverages, including tea, wine, onions, cocoa, cranberries, and apples, have been identified as particularly beneficial in the diet due to their high phenolic component content[5]. Apple fruit (*Malus domestica* Borkh.) from the Rosaceae family is a great source of polyphenols, vitamins, and carotenoids. Antioxidant compounds in apples are quercitin-3-glucoside, catechin, epicatechin, gallic acid, quercitin-3-galactoside, coumaric acid, procyanidin, cyanidin-3-galactoside, and chlorogenic acid[4]. A reduction in the risk of cardio-metabolic disorders, asthma, Alzheimer's disease, colorectal cancer, and lung cancer has been attributed to apple consumption[6].

Numerous studies have developed plant extract recently using nanotechnology. Nanoparticles (NPs), liposomes, and nanoemulsions are the three types of nanosystems most frequently used. Systems based on nanotechnology can be used to improve the biopharmaceutical and technological properties of plant extracts. In addition to the benefits of herbal-based chemicals for wound healing, nanosizing these therapies or incorporating them into nanoparticles gives the opportunity to control their transport to the wounded side and can boost their chemical activity[7]. Researchers have concentrated on formulating plant extract-loaded Nanoparticles (NPs) to combine the advantages of nanotechnology and the variety of biological activities of plant extracts. Due to their size and distinct physicochemical properties, plant extract loaded NPs offer a number of benefits. Additionally, plant extract loaded NPs can be employed to reduce the toxicity of plant extracts, offer targeted drug delivery, and address stability-related issues[8]. So far, there have been no reports describing antioxidant activity of apple fructus extract nanoparticle. The goal of the current study was to examine the antioxidant activity such as DPPH, H_2O_2, and FRAP of apple extract nanoparticle using *in vitro* models.

2. Materials and Methods

2.1 Chemicals

Apple or *M. domestica* fructus extract (PT. FAST, No Batch: 001.10.23.EBA.01), chitosan, glacial acetic acid, propyleneglycol, ethanol 70 %, DMSO, Na-TPP, aquadest, DPPH (*2,2-diphenyl-1-picrylhydrazyl*) (Sigma D9132), Absolute Methanol (Merck 1060092500), Ferrous Ammonium Sulfate (Sigma 7783859), Hydrogen Peroxide (Merck 1.08597.1000), sulfuric acid (Merck 109981), 1,10-phenanthroline (Sigma Aldrich, 131377), routine compound (Chengdu BP1234), protothuic acid compound (Chengdu BP1155), caffeic acid compound (Chengdu BP0300), ferullic acid compound (Chengdu BP0586), and chlorogenic acid compound (Chengdu BP0345).

2.2 Apple Extract Nanoparticle

M. domestica fructus extract was made by PT FAST (No. Batch: 001.10.23.EBA.01) with GMP standards, simplicia (raw material) was soaked with solvent (Ethanol 70 %) for 24 hours. Then the soaking was squeezed and the liquid extract was added with additional materials so that nanoparticles could then be made.

Apple extract nanoparticles were made in the following way: one gram of chitosan was dissolved in 100 mL of 1 % glacial acetic acid using a magnetic stirrer to obtain a 1 % chitosan concentration. A total of 15 ml of apple extract was added with mixed solvent (20 ml of propylene glycol: 20 ml of 70 % ethanol: 20 ml of 10

% DMSO) and 100 ml of aquadest. Then 40 mL of 1 % chitosan solution was added so that the concentration of chitosan became 0.2 %. The mixture was stirred using a magnetic stirrer for 10 minutes. Then it was dripped with 20 mL of 0.4 % Na-TPP at a speed of 1 drop/3 seconds with a burette and in a magnetic stirrer at 300 rpm until nanoparticles were formed which were characterized by homogeneous turbidity. Then stay on the magnetic stirrer for 15 minutes to get a solution of apple nanoparticles, turbidity and sediment[9].

2.3 Characterization of Apple Extracts Nanoparticle

A dynamic light scattering particle size analyzer was used to measure the size of the particles and their zeta potential (Horiba SZ-100). A cleaned cuvette containing around 10 ml of the sample was then put into the sample container for analysis[10].

2.4 Antioxidant Activity Determination by *1,1-diphenyl-2-picrylhydrazyl* (DPPH) Radical Scavenging

Briefly, 50 µl apple extract nanoparticle samples at a range of various final concentrations: 100; 50; 25; 12.5; and 6.25 µg/ml, was placed into a 96 well plate. It was followed by addition of 200 µl 0.077 mmol DPPH (Sigma Aldrich D9132) into the well plate containing the sample (well sample). To the well blank, it was added 200 µl of sample solvent (DMSO). To the well control, it was added 250 µl of 0.077 mmol DPPH. The plate was incubated for 30 minutes in a dark place (room temperature). The absorbance was measured using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, USA) at 517 nm wavelength[11,12]. The percentage of DPPH radical scavenging activity was calculated with the following equation:

$$\%\ \text{DPPH scavenging activity} = 1 - \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

Additionally, the median inhibitory concentration (IC_{50}) for DPPH activity was determined[11,12].

2.5 Antioxidant Activity Determination by Hydrogen Peroxide Scavenging

Briefly, 60 µl apple extract nanoparticle samples at a range of various final concentrations: 200; 100; 50; 25; and 12.5 µg/ml, was placed into a 96-well plate (well sample and well blank). It was followed by addition of 12 µl *Ferrous Ammonium Sulfate* (1 mM, Sigma 7783859) and 3 µl H_2O_2 (5 mM, Merck 1.08597.1000) into the well plate containing the sample (well sample). To the well blank, it was added 90 µl of sample solvent (DMSO). To the well control, it was added 12 µl of *Ferrous Ammonium Sulfate* (1 mM, Sigma 7783859) and 63 µl DMSO[13].

Then, after adding H_2O_2, the control solution mixture, sample, and blank were put into a 96-well plate and incubated for 5 minutes in a dark room at room temperature. Then, 75 µl of 1,10-phenanthroline was added to each sample mixture, then incubated again for 10 minutes in a dark room at room temperature. The absorbance was measured at a wavelength of 510 nm[11,12].

The formula is used to determine the proportion of trapping activity[11,12]:

$$\%\ H_2O_2\ \text{Scavenging Activity} = \frac{\text{A Sample}}{\text{A Control}} \times 100$$

2.6 Antioxidant Activity Determination by FRAP Assay

The FRAP reagent was prepared freshly by mixing 10 mL of acetate buffer 300 mM (pH 3.6 adjusted with addition of acetic acid), 1 mL of ferric chloride hexahydrate (Merck 1.03943.0250, USA) 20 mM dissolved in distilled water, and 1 mL of *2,4,6-Tris(2-pyridyl)-s-triazine* (TPTZ) (Sigma Aldrich 3682-35-7) 10 mM dissolved in HCl 40 mM. A total of 7.5 µl samples of various apple nano concentrations (1000; 500; 250; 125; 62.5 µg/mL) and 142.5 µl of FRAP solution were put into the sample wells and DMSO was added to the blank and control wells. The microplate was closed, and then incubated at 37˚C for 6 minutes. Absorbance measurement used a microplate reader at a wavelength of 595 nm. Standardization with Ferrous sulfate (0.03) gram $FeSO_4$ in 100 mL ddH_2O[11,12].

2.7 Statistical Analysis

Statistical analysis was conducted using SPSS software (version 20.0). Values were presented as Mean ± Standard Deviation. Significant differences between the groups were determined using the Analysis of

variance (One Way ANOVA) followed by Tukey's HSD Post-Hoc Test.

3. Results

3.1 Particle Size Analyzer (PSA) and Zeta Potential Measurement of Apple Extract Nanoparticle

The particle size examination using the Particle Size Analyzer tool with three repetitions, the following results are obtained in Table 1. The particle size average of apple extract nanoparticles is 562.3 ± 461.87 nm (Figure 1).

From the results of the examination of the zeta potential with three repetitions, the following results were obtained in Table 2. The zeta potential average of apple extract nanoparticles was 15.03 ± 0.15 mV (Figure 2).

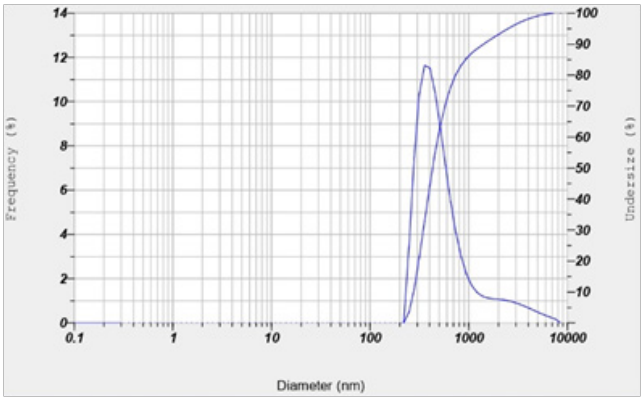
3.2 DPPH Radical Scavenging Activity

This test relies on the creation of a non-radical from 2,2-diphenyl-1-picrylhydrazine, which causes alcoholic DPPH solution reduction in the presence of an antioxidant that donates hydrogen (DPPH-H). The DPPH samples generally changes a purple color into a colourless state when antioxidant molecules quench DPPH free radicals[14]. DPPH scavenging activities of apple extract nanoparticles are shown in Figure 3. The scavenging effect of apple extract nanoparticle on the DPPH radical increased in the following concentration: AN100 (80.35 ± 1.12 %) > AN50 (64.78 ± 0.84 %) > AN25 (54.52 ± 0.87 %) > AN12.5 (50.93 ± 1.11 %) > AN6.25 (46.36 ± 1.50 %). As concentration increased, these samples' free radical-scavenging ability improved.

The results of this study, apple extract nanoparticles have antioxidant activity with an IC_{50} value of 12.16 ± 2.98 µg/ml (Table 3).

3.3 Antioxidant Activity Determination by Hydrogen Peroxide (H_2O_2) Scavenging

The intercellular precursor of hydroxyl radicals, which are extremely harmful to cells, is hydrogen peroxide. It can directly deactivate a small number of enzymes, typically by oxidizing vital thiol (-SH) groups[15]. The ability of apple extract nanoparticle to scavenge



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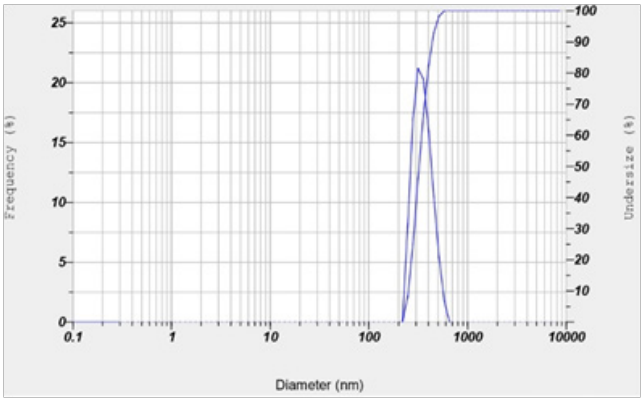

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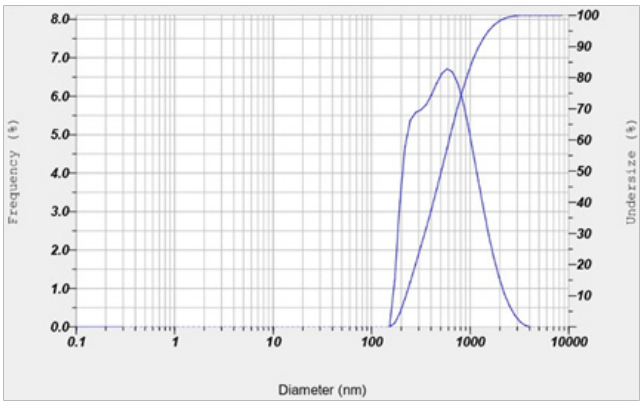

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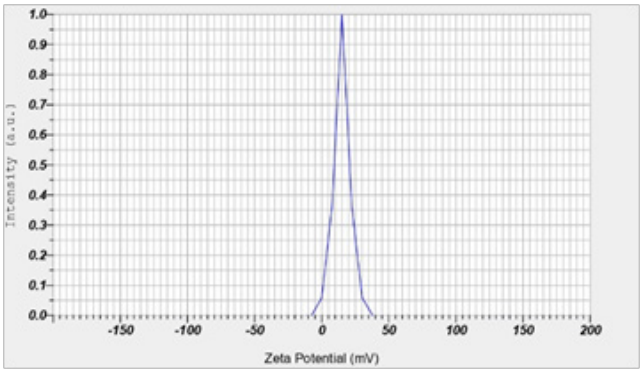
Figure 1. Particle size of apple extract nanoparticle.

Table 1. Nanoparticle size of apple extract

Repetition	Size (nm)
Repetition 1	715.4 ± 868.5
Repetition 2	334.7 ± 72.2
Repetition 3	636.8 ± 444.9
Average	**562.3 ± 461.87**

*Data are presented as Mean ± Standard Deviation



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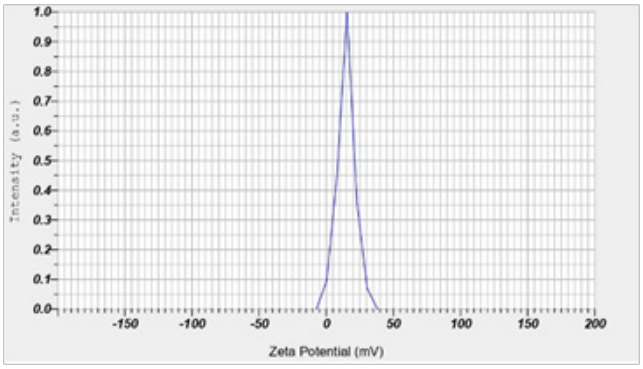

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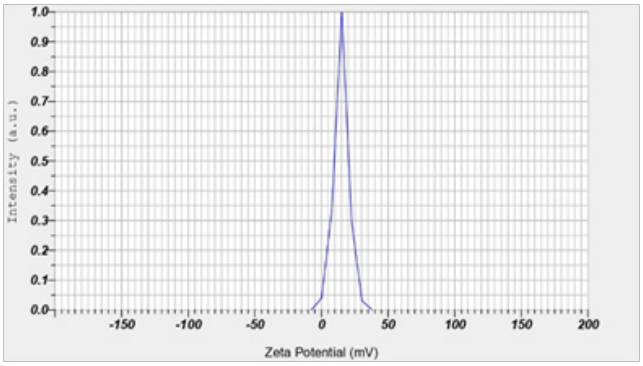

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Figure 2. Zeta potential of apple extract nanoparticle.

Table 2. Zeta potential of apple extract nanoparticle

Repetition	Zeta Potential (mV)
Repetition 1	15.2
Repetition 2	14.9
Repetition 3	15.0
Average	**15.03 ± 0.15**

*Data are presented as Mean ± Standard Deviation

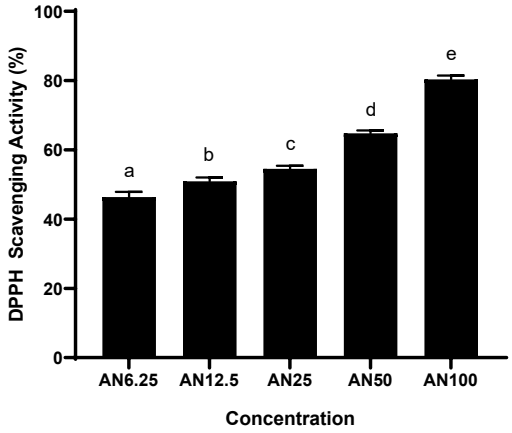

Figure 3. DPPH scavenging activity percentage of apple extract nanoparticle.

*AN6.25: Apple extract nanoparticle 6.25 µg/ml concentration; AN12.5: Apple extract nanoparticle 12.5 µg/ml concentration; AN25: Apple extract nanoparticle 25 µg/ml concentration; AN50: Apple extract nanoparticle 50 µg/ml concentration; AN100: Apple extract nanoparticle 100 µg/ml concentration.

*The data was presented as mean + standard deviation. Statistical differences are generated using Tukey's HSD post hoc test ($p<0.05$). The different letter (a, b, c, d, e) shows significant difference among various concentrations.

Table 3. IC_{50} value of DPPH scavenging activity from apple extract nanoparticle

Sample	Equation	R^2	IC_{50} Value (µg/ml)
Apple Extract Nanoparticle	y = 0.3539 × + 45.675	0.99	12.16 ± 2.98

hydrogen peroxide is shown in Figure 4. These results showed that 200 µg/ml apple extract nanoparticle exhibited most effective hydrogen peroxide scavenging activity than other concentrations. Hydrogen peroxide scavenging activity of these samples enhanced with increasing concentration except 12.5 µg/ml concentration was higher than 25 µg/ml concentration: AN200 (84.47 ± 5.99 %) > AN100 (55.28 ± 1.08 %) > AN50 (41.61 ± 7.05 %) > AN12.5 (32.30 ± 3.88 %) > AN25 (30.43 ± 1.08 %). Based on these results, apple extract nanoparticle has the potential as an antioxidant.

The results of this study showed that apple extract nanoparticles have antioxidant activity with an IC_{50} value of 81.96 ± 7.23 µg/ml (Table 4).

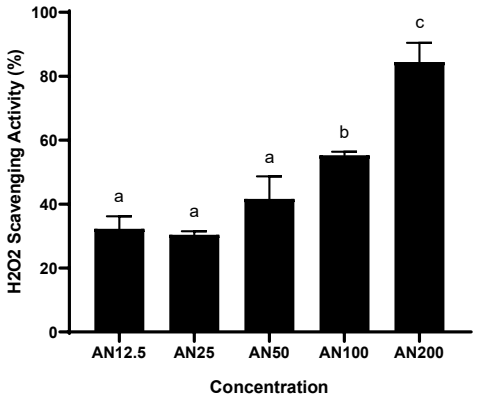

Figure 4. H_2O_2 scavenging activity percentage of apple extract nanoparticle.

*AN12.5: Apple extract nanoparticle 12.5 µg/ml concentration; AN25: Apple extract nanoparticle 25 µg/ml concentration; AN50: Apple extract nanoparticle 50 µg/ml concentration; AN100: Apple extract nanoparticle 100 µg/ml concentration; AN200: Apple extract nanoparticle 200 µg/ml concentration.

*The data was presented as mean + standard deviation. Statistical differences are generated using Tukey's HSD post hoc test ($p<0.05$). The different letter (a, b, c) shows significant difference among various concentrations.

Table 4. IC_{50} value of H_2O_2 scavenging activity from apple extract nanoparticle

Sample	Equation	R^2	IC_{50} Value (µg/ml)
Apple Extract Nanoparticle	y = 0.2903 × + 26.32	0.99	81.96 ± 7.23

3.4 Antioxidant Activity Determination by FRAP Assay

The FRAP technique is based on an antioxidant's capacity to reduce (electron transfer) Fe^{3+} to Fe^{2+} ions in the presence of TPTZ (*2,4,6-tris(2-pyridyl)-s-triazine*), resulting in the formation of a bright blue Fe^{2+}-TPTZ complex[16]. FRAP activity was presented in Figure 5. In this study, the most effective FRAP activity of apple extract nanoparticle is at 50 µg/ml concentration. These samples' FRAP activity increased as concentration increased: AN50 (444.29 ± 17.38%) > AN25 (328.57 ± 19.64%) > AN12.5 (274.29 ± 14.50%) > AN6.25 (222.86 ± 1.43%) > AN3.13 (204.76 ± 10.03). These results indicate 3.13 µg/ml concentration has lowest FRAP activity among samples. Based on FRAP activity, apple extract nanoparticle has antioxidant activity.

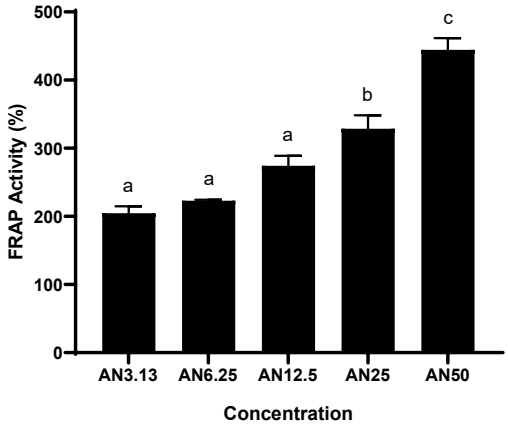

Figure 5. FRAP activity percentage of apple extract nanoparticle.

*AN3.13: Apple extract nanoparticle 3.13 µg/ml concentration; AN6.25: Apple extract nanoparticle 6.25 µg/ml concentration; AN12.5: Apple extract nanoparticle 12.5 µg/ml concentration; AN25: Apple extract nanoparticle 25 µg/ml concentration; AN50: Apple extract nanoparticle 50 µg/ml concentration.

*The data was presented as mean + standard deviation. Statistical differences are generated using Tukey's HSD post hoc test ($p<0.05$). The different letter (a, b, c) shows significant difference among various concentration.

4. Discussions

An organism's natural defense mechanisms, whether they come from enzymatic, non-enzymatic, or nutritional sources, are influenced by the excessive production of reactive oxygen and nitrogen species, which can lead to oxidative damage to macromolecules and tissue damage. Consuming foods (particularly fruits) high in antioxidants is an efficient way to deal with such tissue damage and undesirable changes and can stop the onset of chronic diseases[17].

Regular apple consumption is associated with a range of health benefits since apples are a significant dietary source of carotenoids and phenolic chemicals. Apples contain a variety of phenolic compounds with significant antioxidant qualities, including procyanidins, chlorogenic acids, flavonoids, hydroxycinnamic acids, anthocyanins, and quercetins. Phenolic compounds can be used to treat a number of human conditions, such as cancer, obesity, diabetes, asthma, and cardiovascular illnesses[18]. Apples are a good example of a herbal remedy that is a plentiful source of beneficial components that contain antioxidants and other ingredients that can be used in

foods for certain purposes. This kind of cooperative study between conventional "herbal cures" and more recent methods of the current drug delivery system, i.e., "Nanotechnology," has established appealing pharmaceutical medicines in the near future that will improve people's health. The useful and significant relevance of natural goods and herbal treatments used with the nanocarrier is projected to increase the significance of current drug delivery systems. Therefore, putting "herbal remedy" in nanocarriers will boost its potential for treating different chronic conditions and having positive health effects. There are numerous effective examples and empirical data available to us in the context of nano research[19].

The target of the discipline of applied science and technology known as nanotechnology is to create instruments and dosage forms with a size between 1 and 100 nm. Nanomedicine is the term used recently to describe the use of nanotechnology in the treatment, diagnosis, monitoring, and control of biological systems. The lipids, polysaccharides, and synthetic biodegradable polymers used to create the nanocarriers are all safe substances. The following are the reasons why a nano-sized delivery mechanism was chosen: 1) Their distinct size and high loading capacity appear to enable them to deliver high medication concentrations to disease locations. 2) Drugs should be administered in tiny particles to maximize their surface area and hasten blood clotting. 3) The concentration appears to remain at the locations over the longer times[19]. In this study, evaluation of the total antioxidant activities of the apple extract nanoparticle were estimated using three *in vitro* assays, namely, DPPH, H_2O_2, and FRAP assays. The protective effects of apple extract nanoparticle's biologically active ingredients on inflammatory response are significantly affected by the discovered antioxidant pathways[17].

The Particle Size Analyzer (PSA) is an instrument that measures the size distribution of particles moving with Brownian motion using the dynamic light scattering principle. The velocity of the particles in the medium then coincides with the particle size of the silver nanoparticles. The laser beam illuminated into the sample will experience intensity fluctuations due to particle light scattering[20]. In the present study, apple extract nanoparticles have an average particle size of 562.3 nm with three repetitions (Table 1). This shows that the nanoparticles from apple extract made have met the requirements, where a particle is said to be a nanoparticle if it is in the range of 1-1000 nm[9].

Zeta-potentials for nanoparticles are reasonably simple to measure, and they are frequently suggested as a particle feature that must be included for thorough characterization of nanoparticles in guideline documents[21]. The zeta potential, which is dependent on surface charge, plays a crucial role in the initial adsorption of nanoparticles onto the cell membrane as well as the stability of nanoparticles in suspension. The endocytotic absorption rate after adsorption is influenced by particle size. The zeta potential and size thus affect nanoparticle toxicity[22]. In this study, the zeta potential average of apple extract nanoparticles is 15.03 mV (Table 2). With the similarity of each positive (+) charged particle of apple extract nanoparticles, the repulsion between the particles is getting bigger. This indicates that the nanoparticle suspension is stable so that the possibility of the formation of aggregates from nanoparticles dispersed in the suspension system is reduced. This effect is a result of the lengthy amine group of chitosan's anionic group being bound to a high electrical value in order to prevent aggregation[9].

The DPPH assay is a rather quick and effective way to assess free radical scavenging capacity. It is able to accept an electron or hydrogen radical to form a stable diamagnetic molecule[23]. When neutralized and transformed into DPPH-H, the DPPH radical loses its color and becomes colorless or pale yellow[24]. The sample concentration necessary to block 50% of a radical was estimated using the IC_{50} value. The antioxidant activity of samples increases with decreasing IC_{50} values[23]. Strong antioxidant activity is defined as having an IC_{50} value between 10 and 50 µg/ml, moderate antioxidant activity as being between 50 and 100 µg/ml, and weak antioxidant activity as being greater than 100 µg/ml[25]. Based on the results of this study, apple extract nanoparticles have a strong antioxidant activity with an IC_{50} value of 12.16 µg/ml (Table 3). This result indicates that apple (*Malus domestica*) fructus extract nanoparticles have the ability to donate more electrons to DPPH than the ethanol extract of apple (*Malus domestica*) peel waste (IC_{50} = 87.795 µg/ml)[26] and freeze-dried fruit sample of *Malus domestica* (805.11

µg/ml)[27]. The difference in IC_{50} value is caused by the ability of each compound to donate electrons to DPPH, the more electrons given to DPPH will result in a decrease in the absorbance value which means an increase in the percentage of inhibition and a decrease in the IC_{50} value[26].

Hydroxyl radical scavenging activity of apple extract nanoparticle is shown in Figure 4. In this study, there was a consistent increase in concentration in H_2O_2 scavenging activity of apple extract nanoparticle. The IC_{50} value of H_2O_2 assay was calculated to scavenge 50% of the peroxide in a system. It is used to evaluate the antioxidant capacity of apple extract nanoparticle (Table 4). The lower the IC50, the better the antioxidant potential of the sample under examination. Based on IC_{50} value on H_2O_2 assay, apple extract nanoparticle has intermediate antioxidant activity (IC_{50} = 81.96 ± 7.23 µg/ml). Many oxidizing enzymes, including superoxide dismutase, which can traverse membranes and slowly oxidize a variety of substances, can produce hydrogen peroxide in living organisms[28]. The hydroxyl radical induces severe damage to adjacent biomolecules such as lipids, proteins and DNA[29]. According to this study, apple extract nanoparticles are good scavengers of hydroxyl radicals and may employed in such conditions.

The FRAP activity method is based on the reduction of a ferroin analogue, the Fe^{3+} complex of tripyridyltriazine $Fe(TPTZ)^{3+}$ to the intensely blue coloured Fe^{2+} complex $Fe(TPTZ)^{2+}$ by antioxidant in acidic medium. Antioxidant substances that function as reducing agents do so by adding a hydrogen atom to the ferric complex, which stops the radical chain reaction[5]. FRAP activity can be used to determine antioxidant capacity of apple extract nanoparticle (Figure 5). Through this assay, it is indicated that the apple extract nanoparticle at 50 µg/ml have higher reducing power (444.29 %) to react with ferric tripyridyl triazine (Fe^{3+}-TPTZ) complex and yielded a final product of ferrous tripyridyl triazine (Fe^{2+}-TPTZ) as compared to other concentrations[30].

5. Conclusion

Nanoparticles of apple extract could be formulated and the average particle size of apple extract nanoparticle was 562.3 nm. The average zeta potential of apple extract nanoparticles was 15.03 mV. The apple extract nanoparticle has strong antioxidant activity based on DPPH, H_2O_2, and FRAP assay. The rise of concentration affects the antioxidant activities.

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