



THE REDUCING POWER AND DPPH FREE RADICAL SCAVENGING ACTIVITY
OF POLYPHENOLS FROM ETHANOL EXTRACT OF *ALLIUM CEPA* L. LEAVES
AND PEEL

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Abstract

Onions have a wide range of uses in the chemical and food industries including increasing product, as ingredients in food, substrate for enzyme production, and as colourant in some dishes. The study evaluated the tannin, flavonoids, and phenolic contents of the ethanol extract of onion peel and leaves as well as the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power. The total antioxidant capacity, flavonoids, tannins, and phenol contents of the extracts were assessed using standard methods. The In-vitro antioxidant activities (reducing power activity and DPPH free radical scavenging activity) were also estimated using standard methods. The results showed that the total phenol and tannins content of the onion leaf extracts (258.00 ± 6.00 and 254.70 ± 6.03) was significantly higher ($p < 0.05$) compared to the onion peel extract (110.70 ± 1.20 and 67.33 ± 4.04). Conversely, the total flavonoid content of the onion peel extract (1.30 ± 0.02) was significantly higher ($p < 0.05$) relative to the onion leaves extract (0.38 ± 0.02). One-way ANOVA revealed a non-significant ($p > 0.05$) increase in the total antioxidant activity of onion leaf extract (0.63 ± 0.03) compared to the onion peel extract (0.53 ± 0.03). Conclusively, the ethanol extract of onion leaves and peel contains varying amounts of tannins, phenols and flavonoids with strong antioxidant properties. These compounds can be extracted from onion leaves and peels that are usually discarded to serve as antioxidants for improving livestock and human health.

Keywords: Antioxidant, flavonoids, onion peels, onion leaves, phenols, tannin

Introduction

Oxidative stress occurs when the cellular and extracellular antioxidant capacity is superseded by lipid peroxidation resulting in the generation of free radicals (Arias et al., 2022). Oxidative stress is believed to promote the progress of many diseases such

as diabetes, cardiovascular disease, neurodegeneration and metabolic disorders (Asgard et al., 2007; Dehimat et al., 2021; Dibal et al., 2022). Consumption of fruits and vegetables is the most effective way of preventing oxidative stress as well as slowing the progress of many diseases (Arias et al., 2022). The protective effects

of fruits and vegetables against many diseases are attributed to their phytochemistry and antioxidant properties (Wang et al., 2022a; Girgis et al., 2024).

Onion (*Allium cepa* L.) is an old vegetable grown in Africa, parts of America, Asia, and Europe. It is the second most common cultivated and consumed plant worldwide (Kavalcova et al., 2015; Chernukha et al., 2021). The global production of onion is estimated at 90 million tons per year with China, India, Egypt, USA and Iran rated as the top five producers (Kumar et al., 2022). Onions have a wide range of uses in the chemical and food industries. This include extending the shelf-life of products during packing (oil stability), serving as ingredients in food items such as bread, noodles, sausages, cake, and meat, acting as a substrate for enzyme production, and functioning as a colourant in some dishes (Wiczowski, 2011; Ali et al., 2016; Lorendana et al., 2019; Sagar et al., 2022). Reports from previous studies showed that onions have neuroprotective (Chernukha et al., 2021), in-vivo and in-vitro antioxidant activities (Kim et al., 2011; Umeda and Jorge, 2023), antimicrobial and anticancer properties (Kim et al., 2011; Nile et al., 2021). The biological activities of onions are attributed to the presence of flavonoids, polyphenols, anthocyanins and quercetin (Skerget et al., 2009). Onions are believed to exert their biological activities by acting as antioxidants and preventing lipid oxidation, oxidative stress and inflammation (Kim et al., 2022). The quercetin and phenols present in onions are reported to be higher in the outer onion skin and leaves compared to the bulb (Skerget et al., 2009). The fresh leaves have a short life and are usually discarded before taking the onions to the market, storage and further

processing of the bulb also result in the generation of waste mainly from the outer peels, roots and damaged bulb (Yuasa et al., 2020; Kumar et al., 2022). The waste generated from onion promotes the growth of microorganisms (phytopathogens) and has a strong aroma/smell that makes it unsuitable for land disposal (Singh et al., 2017). The waste can be used for the production of fertilizers, supplemented in animal feed production as well as the extraction of biologically active ingredients for the prevention and treatment of many diseases (Horiachi et al., 2004; Cavaleiro et al., 2021). Hence, the present study evaluated the tannin, flavonoids, and phenolic contents of the ethanol extract of onion peel and leaves as well as the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power.

Methodology

Reagents

All the reagents used were of analytical grade and were either purchased from Sigma-Aldrich, USA, Merck, Germany or Shandong Shuowan Chemical Co. Ltd, China. They include aluminium chloride, ascorbic acid, quercetin, Folin-Ciocalteu reagent, sodium phosphate buffer, ethanol, potassium ferric cyanide, Sodium nitrite, sodium hydroxide, polyvinyl pyrrolidone, ferric chloride, Gallic acid, and 1,1-Diphenyl-2-picrylhydrazyl (DPPH).

Plant identification and extraction

The onion peels and leaves were purchased from Gamboru market, Maiduguri, Nigeria and authenticated and deposited at the Herbarium in the Faculty of Pharmacy, University of Maiduguri, Nigeria (Voucher no:

UMM/FPH/AMA/001). The leaves were washed, blended and dissolved in absolute ethanol (1:1 ratio) while the peels were ground and dissolved in absolute ethanol (1:1 ratio). The mixtures were left for 48 hours with constant shaking. It was filtered and evaporated in an oven at 45 ° C to get the extracts.

Phytochemical analysis and in-vitro antioxidant activities

The total phenol, tannin and flavonoid content were evaluated as described in our previous study (Manye et al., 2023), total antioxidant capacity was determined according to the methods of Hossen et al. (2022), DPPH radical scavenging activity was determined according to the method described by Blois (1958) with some modifications while the reducing power activity was determined as described by Yildirim and Kara (2001).

Total phenol content

Twenty micrograms of the extract were mixed with distilled water to make 1 mL. 20% sodium carbonate (2.5 mL) and distilled water plus 500 µL of Folin-phenol reagent (1:1 ratio) were added to the mixture and shaken vigorously. The mixture was incubated in the dark for 40 min to develop color and absorbance was measured at 725 nm. A gallic acid standard calibration curve was created and the linearity was established. Total phenol was expressed as mg of Gallic acid equivalent (mg GAE/g extract) using the curve.

Tannin content

Five hundred microliters of the extract were mixed with 500 µL of distilled water and 100 mg of polyvinyl pyrrolidone. The mixture was incubated for 4 hours at 4 °C and centrifuged at 5000 rpm for 5 min. The

phenol content was measured at 725 nm and expressed as free phenols on a dry matter basis. The tannin content (mg GAE/g extract) was calculated as Total phenols (mg GAE/g extract)-Free phenols (mg GAE/g extract).

Total flavonoid content

Two hundred millilitres of distilled water were added to 1 mL of the extract. Then, 150 µL of 5% five per cent sodium nitrite (150 µL) was added and the solution was incubated for 5 min. 150 µL of 10% aluminium chloride was added and allowed for 6 min. Then, 2 mL of sodium hydroxide (4%) and distilled water were added to make 5 mL. The mixture was vigorously shaken and allowed for 15 min at room temperature. Absorbance was spectrometrically measured at 510 nm and total flavonoid content was expressed as mg of quercetin equivalent (mg QE/g extract) on a dry weight basis using the standard curve.

Total antioxidant capacity

Three millilitres of the reagent (4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid) were added to 1 ml of the extract. The solution was incubated at 95 ° C for 1 hour 30 min and allowed to cool at room temperature. The absorbance of the solution was measured at 695 nm against a blank (1 ml ethanol). The antioxidant activity was expressed as milligrams of Gallic acid equivalents.

Reducing power activity

Freshly prepared 1% potassium ferric cyanide (1 mL) 0.2 mol/L sodium phosphate buffer (1 mL) at pH 6.6 were added to different concentrations (300-700 µg/mL) of the extract in triplicate. The

solution was incubated in a water bath for 20 min at 50 °C and 10% trichloro acetic acid (1 mL) was added. The solution was centrifuged at 3000 rpm for 10 min and the supernatant was mixed with distilled water (1:1 ratio). Freshly prepared 1% ferric chloride (500 µL) was added to the mixture and absorbance was read at 700 nm. The results were compared with standard antioxidants (ascorbic acid).

DPPH free radical scavenging activity

About 0.2 mmol/L solution of DPPH in distilled water and ethanol was prepared. The solution (500 µL) was added to different concentrations (50-250 µg/ mL) of the extracts. The mixture was agitated and allowed to settle at room temperature for 30 min. The same procedure was used to prepare a control (no extracts, distilled water and ethanol) for baseline correction. The changes in the absorbance at different concentrations of the extracts were measured at 517 nm. Results were compared with ascorbic acid (standard antioxidant). The DPPH scavenging effect (percentage inhibition) = (Absorbance of control-Absorbance of sample/Absorbance of control) x100.

Statistical analysis

GraphPad Prism version 9 (GraphPad Software) was used to analyze the data. Student t-test was used to compare the levels of flavonoid, tannins and total phenols between the two extracts while one-way ANOVA was used to compare the total antioxidant capacity of the two extracts with ascorbic acid. Statistical significance was considered at $p < 0.05$.

Results

Phytochemical analysis

The result of the student t-test showed that the total phenol and tannins content of the onion leaf extract (258.00 ± 6.00 and 254.70 ± 6.03) was significantly higher ($p < 0.05$) compared to the onion peel extract (110.70 ± 1.20 and 67.33 ± 4.04). However, the total flavonoid content of the onion peel extract (1.30 ± 0.02) was significantly higher ($p < 0.05$) relative to the onion leaves extract (0.38 ± 0.02) (Figure 1). One-way ANOVA revealed a non-significant ($p > 0.05$) increase in the total antioxidant activity of onion leaf extract (0.63 ± 0.03) compared to the onion peel extract (0.53 ± 0.03). The total antioxidant capacity of the two extracts was significantly lower relative to the standard (ascorbic acid) with (2.05 ± 0.03) at $p < 0.05$ (Figure 2). The total antioxidant capacity of the onion leaf extract was equivalent to 30.7 % of the standard (ascorbic acid) while that of the onion peel extract was equivalent to 25.9 %.

In-vitro antioxidant activities

The DPPH free radical scavenging activity of the onion peel and leaves extracts was expressed as % inhibition and decreased with increasing concentration from 50 µg/ml to 250 µg/ml. The % inhibition ranged from (84.33 % to 95.33 %) in onion peel and (65.60 % to 84.17 %) in onion leaves. The percentage inhibition for the onion peel extract was significantly higher ($p < 0.05$) compared to the onion leaf extract (Figure 3). For the reducing power activity, the absorbance of both the onion peel and leaves increases as the dose increases from 300–700 µg/ml. However, the absorbance of the onion peel extract ($0.19-0.33$) was significantly lower relative to the onion leaf extract in all the doses ($0.56-1.30$) at $p < 0.05$ (Figure 4).

Discussion

The phytochemical screening showed that the onion leaf extract had a higher phenol and tannin content compared to the peel extract. Phenols are reported to aggregate more on the skin and leaves of fruits and vegetables because of their ability to protect against predators, pathogens and UV rays (Pablo et al., 2022). The higher phenol content in onion leaf extract that was observed in the present study suggests that the leaves have a better capacity to protect against pathogens, UV rays and predators compared to the peel. Additionally, the leaves of onions are exposed to the external environment from planting to harvest while the bulb that is usually surrounded by the peel is buried in the ground and only exposed during transportation and storage. Hence, it might not require as much phenol as the leaves coupled with the fact that they are normally stored in a pathogen-free environment away from sunlight. A previous study reported the phytochemical components and antioxidant activities of some varieties of onion peel extract and found out that the extracts contain high amount of flavonol and anthocyanins with strong antioxidant antiproliferative properties (Fredotovic et al., 2021). Another study evaluated the proximate and phytochemical analysis of two onion peel varieties and the result showed high amount of carbohydrate, crude protein, tannin, and phenol suggesting differences in the phytochemical component of different onion varieties and parts (Sule et al., 2025). Phenolic compounds constitute a major component of the cell wall in plants; they inhibit seed germination and stimulate in-vitro cell division. These suggest that they play an important role in the regulation of plant

growth and development (Babenko et al., 2019). We suggest that onion leaf extract could be an important source of compounds for use in research associated with cell growth, differentiation, and development. Tannins are polyphenols widely found in fruits and vegetables. Appropriate amounts of tannins are reported to improve feed efficiency, animal health and meat quality by substantially increasing the colour stability of muscles, increasing serum superoxide dismutase level and decreasing serum malondialdehyde, cholesterol and low-density lipoprotein levels (Aboagye et al., 2019; Chen et al., 2019). It was also reported to increase antibody titre against some viral vaccines like Newcastle disease (Farahat et al., 2017). However, high concentrations of tannins in plants are believed to affect palatability thereby reducing feed intake. It also reduces digestibility, and nutrients when taken in high quantities (Tong et al., 2022). We postulate that the high content of tannins found in onion leaves might be responsible for low or no consumption by animals as a result of reduced palatability and digestibility.

The current study revealed that the flavonoid content of the onion peel extract was significantly higher compared to the onion leaf extract. Flavonoids are reported to prevent oxidative stress and act as growth regulators (Kumar and Pandey, 2013). They are also responsible for colour display in plants, besides their anti-inflammatory and radical scavenging properties (Panchey et al., 2016). This suggests that onion peel extract acts as a natural antioxidant and anti-inflammatory agent. Hence it can be used for the extraction of compounds especially flavonoids instead of disposal which often

lead to environmental pollution and health hazards. The total antioxidant capacity of the two extracts was not significantly different and their total antioxidant activities were 26% (onion peel) and 31% (leaves extracts) compared to the standard (ascorbic acid). This is an indication that the total antioxidant capacity of the two extracts is dependent on the synergist effect of the various compounds present. We hypothesized that the antioxidant capacity of onion peel and leaf extracts is attributed to the synergism between tannins, phenols and flavonoids. A previous study reported that a combination of antioxidants causes several interactions including antagonism, synergism, and additive effects. Antagonism leads to a reduction in antioxidant activity, synergism increases antioxidant activity, while additive effect neither decreases nor increases the antioxidant activity (Bayram and Decker, 2023). Since synergism is believed to enhance the antioxidant capacity of different compounds (Mrid et al., 2022), extracts containing compounds such as tannins, flavonoids, and phenols could produce better antioxidant and free radical scavenging activities compared to a single compound i.e., either tannin, flavonoids and phenols acting alone. The time of extraction, the solvent used and extraction procedures were all reported to affect the phytochemical constituents of extracts (Hossain et al., 2018). This suggests that solvent type, extraction time and methods play a key role in producing phytochemicals. Hence, the content of compounds present in an extract will depend on it. Even though the same solvent, method and type of extraction were employed in the current study, the different parts used (leaves and peel) are responsible

for the varying concentrations of the phytochemicals present.

In the current study, while the DPPH radical scavenging activity of the onion peel extract was significantly higher compared to the leaves, the reducing power activity of the onion leaves extract was significantly higher compared to the peel. This suggests that the onion peel extract can react with DPPH to produce DPPH-H and reduce lipid peroxidation at a faster rate compared to the onion leaf extract. A previous study reported a percentage inhibition of 57.30% for onion extract and 81.67% for onion peel extract (Wang et al., 2022b). The DPPH activities of onion peel and leaf extracts are attributed to the presence of compounds such as flavonoids and phenols that were reported to be abundant in onion by other researchers (Lee et al., 2015; Sagar et al., 2020). The DPPH free radical scavenging activity of a compound is the compound's ability to release electron which will neutralize DPPH to form DPPH-H and prevent lipid peroxidation/reducing free radical scavenging activity (Manye et al., 2023). DPPH radical scavenging activity was reported to vary in different onion cultivars with percentage inhibition ranging from as low as 5.71% in Bhima Shubhra and 9.79% in Pusa Riddhi to as high as 89.99% in Hissar-3 and 94.17% in NHRDF Red (Sagar et al., 2020). This suggests that the DPPH activity may vary due to species and conditions for growth and development such as soil type, temperature, and geographical location among others.

Compounds with high reducing power activities are believed to exhibit stronger antioxidant activities compared to compounds with lower reducing power activities (Cheng and Li, 2004). Therefore,

the higher reducing power activity of onion leaf extract compared to the peel that was observed in the current study might be because the onion leaf extract has a better chance of denoting electrons that will react with free radicals to prevent oxidative stress. A previous study reported strong reducing power activity of 0.37-0.94 in violet de galmi and 0.27-0.71 in Goudami varieties of onion bulb (Dimitry et al., 2021). Another study reported that polyphenols (phenols, flavonoids, and quercetin) present in various red onion extracts/fractions are responsible for biological activities (Singh et al., 2009; Hossain et al., 2018). This suggests that different species/varieties of onion possess different biological activities and extraction procedures/solvents used can produce extracts with varying amounts of phenols, flavonoids, and quercetin as well as different degrees of antioxidant activity. The different methods of evaluating the in-vitro antioxidant activity of compounds/extracts use different principles, and a particular compound may prove efficient with one method compared to others (Koncic and Jug, 2011). Therefore, the differences in the in-vitro antioxidant activities of the onion leaves and peel extracts suggest that both extracts can serve as a good antioxidant since each extract is more efficient compared to the other in the different methods. Onions are processed for food without including their peels. The leaves are seasonal products that are abundant at the time of harvest with a significant amount discarded as waste. Hence, both onion peel and leaves are

usually underutilized for domestic and industrial use (Kyei et al., 2024). Previous studies have demonstrated that onion byproducts constitute a significant portion of agricultural waste generation millions of tones globally (Crnivec et al., 2021). The waste generated can be utilized by the chemical, drugs, and food industries for the extraction of bioactive compounds. This will address the need of onion waste disposal by utilizing it as a raw material for food and drugs (Celano et al., 2021).

This study has some limitations. Only the flavonoids, tannins, and phenols contents of the extracts, as well as the total antioxidant capacity, reducing power and DPPH free radical scavenging activities, were estimated. We could not evaluate High-Performance Liquid Chromatography (HPLC) and Ferric Reducing Antioxidant Power Assay (FRAP). Thus, the conclusion is based on the assessed parameters.

Conclusions

The findings of the study have shown that the ethanol extract of onion leaves and peel contains varying amounts of tannins, phenols and flavonoids with strong antioxidant properties. Therefore, these compounds can be extracted from onion leaves and peels that are usually discarded to serve as antioxidants for improving livestock and human health. Hence, onion byproducts are important products that can serve as raw material in the food and drugs industries for the production of bioactive compounds in food, chemicals, and drugs.

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Figures

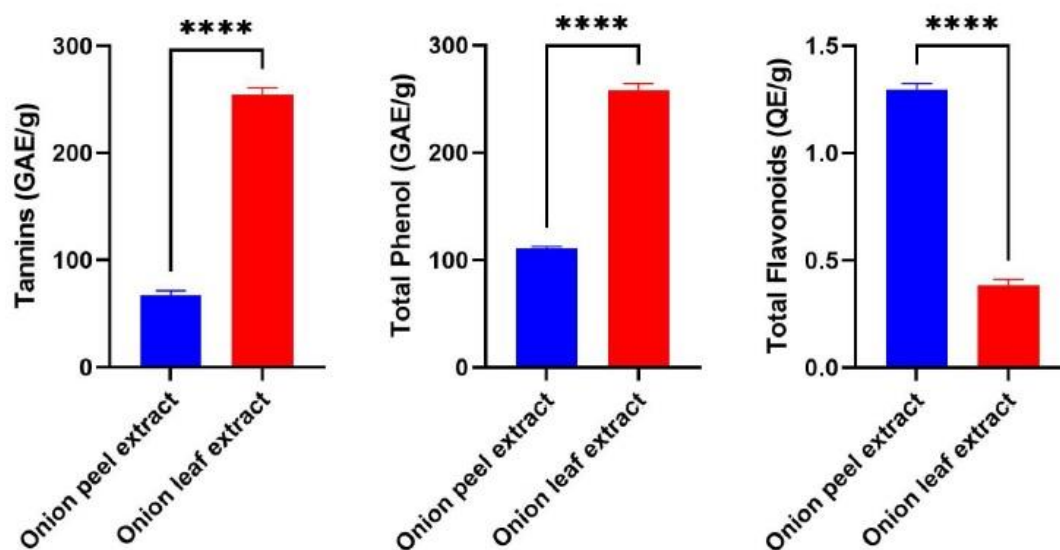


Figure 1: The tannins, total phenol and total flavonoid contents of ethanol extract of onion peel and leaves. The tannin and phenol content are significantly higher in the leaves compared to the peel while the flavonoid content was significantly higher in the peel compared to the leaves. Data presented as Mean±SD. **** indicate significant difference at $p < 0.05$.

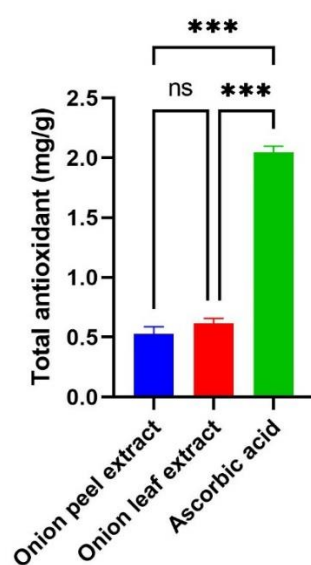


Figure 2: The total antioxidant activity of the ethanol extract of onion peel and leaves. Ascorbic acid had a significantly higher antioxidant activity compared to both extracts. The total antioxidant activity of onion peel and leaf were not significantly different. Data presented as Mean±SD. *** indicates a significant difference at $p < 0.05$ while ns indicates a non-significant change.

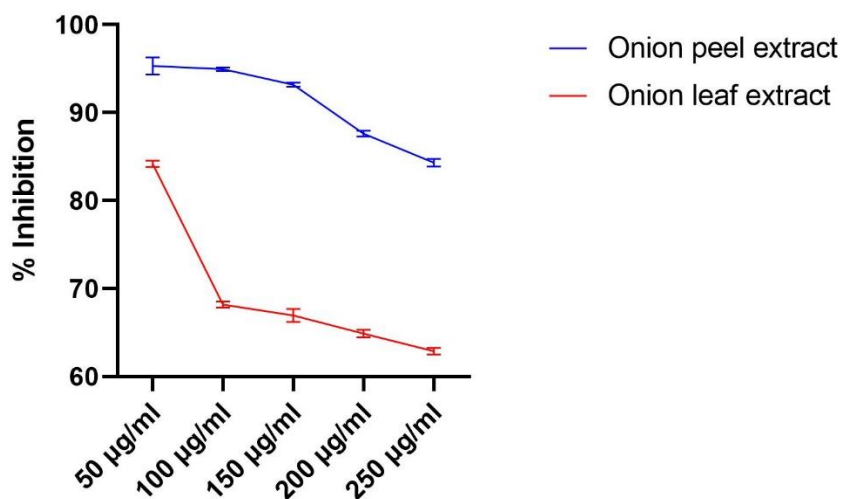


Figure 3: The percentage inhibition for DPPH radical scavenging activity of ethanol extract of onion peel and leaves. Percentage inhibition decreases with increasing dosage. Onion peel had a higher % inhibition compared to the leaves demonstrating that the peel reacts with DPPH to produce DPPH-H and reduce lipid peroxidation better than the onion leaf extract. Data presented as Mean±SD.

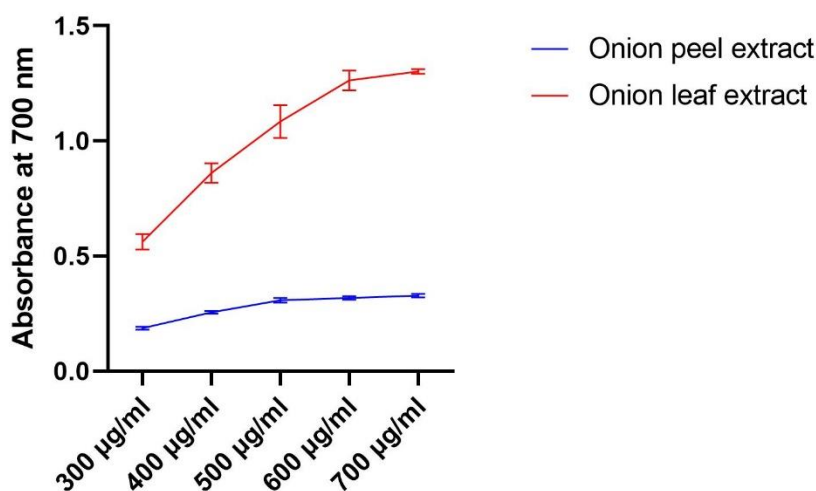


Figure 4: The reducing power activity of ethanol extract of onion peel and leaves. The absorbance increases with increasing dosage. Onion leaves had a higher absorbance rate compared to the peel indicating that the leaves have stronger antioxidant activity compared to the peel. Data presented as Mean±SD.