

Comparative Assessment of Free Radical Scavenging and Reducing Power in Avocado (*Persea americana*) and Papaya (*Carica papaya*) Seed Oils

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Abstract

Reactive oxygen species (ROS), a typical byproduct of aerobic respiration, comprises both the free radical species and non-radical species, and are essential for cell signalling. But when these ROS are present in excess, they can cause oxidative stress, which damages cells. However, antioxidants counteract the negative effects of ROS by preventing or delaying oxidation. Thus, the antioxidant properties of papaya and avocado seed oils were studied in this work using *in vitro* models. Fresh fruits of papaya and avocado were collected, the seeds harvested, and the oils extracted using conventional techniques. The capability of the oils to scavenge ROS was examined through standard methods. The ROS scavenging activities of seed oils was concentration dependent. At 80 mg/ml, papaya and avocado seed oils had the greatest radical scavenging capabilities in DPPH experiment, with $94.829 \pm 0.010\%$ and $85.057 \pm 0.103\%$ inhibition respectively. Similarly, papaya and avocado seed oils exhibited the highest ABTS scavenging activity at 300 mg/ml, with corresponding inhibitions of $71.670 \pm 0.423\%$ and $59.091 \pm 0.317\%$. Again, superoxide scavenging activity results indicate that when increasing papaya seed oil from 10 to 20 mg/ml leads to increased activity from $72.133 \pm 0.344\%$ inhibition to $74.699 \pm 0.402\%$ inhibition. However, when concentration is increased further from 40mg/ml to 80 mg/ml, the activity decreases. Finally, the findings revealed that the ferric reduction antioxidant capacity of the oils increases as concentration increases. The results in this study show that the seed oils demonstrated potent ferric reducing antioxidant capacity and free radical scavenging activities, thus, contributing to the inhibition or delay of the development and progression of several diseases associated with oxidative stress. Consequently, the findings in this study portrays the ability of the oils, especially papaya seed oil, to contribute to ameliorating diseases associated with oxidative stress, developing advanced biomaterials, and acting as essential preservatives in food and pharmaceuticals.

Keywords: Antioxidant, *Persea americana*, *Carica papaya*, reactive oxygen species, radical scavenging activity, ferric reducing antioxidant property.

Introduction

Oxygen radicals are chemical species such as hydroxyl radical ($\cdot\text{OH}$), superoxide anions ($\cdot\text{O}_2$), or their metabolites (hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2)) constantly formed during aerobic respirations (Umerie and Ekuma, 2016) and enzymatic processes involving cyclooxygenase, lipoxygenase, and xanthine oxidase (Cross *et al.*, 1987). These molecular species, due to their capability to harm metabolic molecules, are connected to various diseases, such as benign prostatic hyperplasia, cancers, cardiovascular diseases, diabetes, and neurodegenerative diseases (Olszowy-Tomszyk, 2020; Yap *et al.*, 2023).

Studies suggests that the human body have evolved mechanisms of resisting the effects of these free radicals through the activities of antioxidant enzymes (Harris *et al.*, 1992; Zhang *et al.*, 2015) which function by acting as electron donors. Again, due to the insufficiency of these natural antioxidant mechanisms in combating ROS resulting from daily oxidative stress, researchers developed commercial antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) to aid this function (Yap *et al.*, 2023). Nevertheless, stricter regulations have been imposed on the use of BHA and BHT due to their cytotoxic and carcinogenic effects, and these have led to the increasing demand for plant-based antioxidants to replace them (Nickavar and Esbati, 2012). Plant-based antioxidants are favoured over synthetic ones due to their numerous modes of action, non-toxicity, and few adverse effects (Cross *et al.*, 1987; Yap *et al.*, 2023).

Papaya (*Carica papaya*), of Caricaceae family, grows globally (Almora *et al.*, 2004; Canini *et al.*, 2007). This tree-like plant produces fruits which vary from yellow to orange to reddish, and have numerous seeds organized in rows to the anterior of the fruits (Yanty *et al.*, 2014). Although the seeds are typically thrown away as garbage, some US States, including Hawaii, use them as black pepper and as an ingredient in salad dressing (Jagtiani *et al.*, 1988; Paradkar *et al.*, 2001). The seed of this plant has been described in ethnomedicine to serve as post-testicular

anti-fertility drug and as anti-microbial agents (Lohiya *et al.*, 2000; Gnanamangai *et al.*, 2022). Additionally, research shows that the primary phytochemical in papaya seeds that is responsible for the plant's documented anti-cancer properties is benzyl glucosinolate (Alotaibi *et al.*, 2017; Anilkumar and Bhanu, 2022).

Conversely, Avocado or alligator pear (*Persea americana*), a Central American tree, has gained significant importance in nutrition because of the fruits' high proteins, fat, fibre, vitamin, and mineral content (Oluwole *et al.*, 2013; Maitera *et al.*, 2014; Ahmed *et al.*, 2022; Hernandez-Martinez *et al.*, 2022). Similarly, the seeds of avocado are typically discarded as waste once the fruits are eaten. However, ethnomedicine has documented that the seed decoctions of avocado are utilized in the cultures of Maya and Aztec to treat digestive disorders, inflammations, and parasitic diseases (Dabas *et al.*, 2019). Phytochemical analysis show that avocado seeds are enriched with polyphenols and triterpenoids (Abubakar *et al.*, 2017).

Therefore, this work was targeted at evaluating the capability of papaya and avocado seed oils to scavenge ROS. This was achieved through studying the DPPH, ABTS, superoxide, and hydrogen peroxide radical scavenging activities, as well as ferric reducing antioxidant properties (FRAP). Each of these tests only covers a small percentage of the plants' total antioxidant capabilities because of their unique advantages and disadvantages.

Materials and methods

Plant seeds collection and preparation

Papaya and avocado's ripe fruits were obtained separately from trees growing in the Amangballa community of Afikpo LGA, Ebonyi State, Nigeria. The seeds were extracted, identified, and confirmed by a taxonomist at Ebonyi State University. The voucher numbers are EBSUH-1279 and EBSUH-1479 for papaya and avocado respectively and the specimens placed in the herbarium of the laboratory of the university. Furthermore, a running water was used to clean the seeds before being dried for two weeks in a shed. The parched seeds were separately pulverised into powder with a corona manual pulveriser and kept in a cool, dry location.

Extraction of oils

The oils were extracted from the pulverized papaya and avocado seeds using the solvent extraction process outlined in Pena *et al.* (1992). A cellulose paper cone was used to wrap 50 g of the seed flour and extracted for 16 h using ethanol (b.p. 60 – 80 °C) in a 5-1 Soxhlet extractor. The extracted oils were weighed and recovered with a rotary evaporator. Residual solvents were removed through drying in an oven (60 °C; 1 h) and flushing nitrogen (99.9 %). The oil yield was computed as % sample dry weight.

Antioxidant assay

DPPH scavenging activity.

The procedure described by Mensor *et al.* (2001) was employed in assessing the DPPH scavenging activity of the oils. Methanol (0.48 ml) and 0.1 mM methanolic solution of DPPH (0.5 ml) were combined with the samples (20 µl) and left to react at 20 °C for 30 mins. The reference was butylated hydroxytoluene (BHT), the blank was methanol, and the positive control was DPPH in methanol lacking the oil. An incubation of the mixture was carried out for 30 mins and absorbance (A) calculated at 518 nm with a spectrophotometer.

$$\% \text{ Scavenging of DPPH} = \frac{100 - A(\text{sample}) - A(\text{blank})}{A(\text{blank})} \times 100$$

ABTS scavenging effects.

ABTS was assayed with the procedure of Shirwaikar *et al.* (2006). ABTS solution (7mM) and 2.45mM (NH₄)₂S₂O₈ were mixed to give radical cation ABTS⁺, which was stored for 12 h to 16 h in the dark at 20 °C before use. An aliquot (0.5 ml) of the sample was included in 0.3 ml of ABTS solution, and ethanol used to augment the volume to 1ml. Absorbance was read at 745 nm, and the % inhibition calculated as:

$$\% \text{ Scavenging of ABTS} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Superoxide scavenging activity.

The Winterbourn *et al.* (1975) approach was used to study superoxide anion scavenging activity. 0.02 ml of the seed oil (20 mg) was mixed with 0.2 ml of EDTA, 0.1 ml of nitroblue

tetrazolium, and 0.05 ml of riboflavin. 2.64 ml of phosphate buffer was added to make it up to 3 ml by the addition of. Dimethyl sulphide (DMSO) was introduced to the control tubes in place of the samples. After vortexing, the initial OD of each tube was detected at 560 nm using a spectrophotometer. For 30 mins, a fluorescent light was used to illuminate the tubes before measuring the final OD at 560 nm. SO scavenging activity was calculated as the difference in initial OD and final OD.

H₂O₂ scavenging activity.

H₂O₂ scavenging activity for was performed using the approach of Ruch *et al.* (1989). A 40 nMH₂O₂ solution was created by adding the sample (10 mg/10 µl) to 0.6 ml of H₂O₂ solution, and the volume augmented to 3 ml using phosphate buffer. A blank solution was made with phosphate buffer and no H₂O₂. The absorbance (A) was read at 230 nM using a spectrophotometer at and the H₂O₂ scavenging activity calculated thus:

$$\% \text{ Scavenging of hydrogen peroxide} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$$

Ferric reducing antioxidant properties (FRAP)

The procedure of Pulido *et al.* (2000) was employed in studying FRAP. The extract was mixed with 1 % potassium ferrocyanide (0.25 ml) and 0.25 ml of 200 mM sodium phosphate buffer (pH 6.6). At 20 mins incubation period (50 °C), 10 % trichloroacetic acid (0.25 ml) was added, and centrifuged for 10 min at 2000 rpm. Distilled water (1 ml) was included to the supernatant (1 ml) followed by FeCl₃ (0.2 ml). Absorbance (A) was measured at 700 nm.

Statistical analysis

Data obtained in this study are presented as mean ± SD. The mean differences between more than two groups were ascertained using ANOVA, which was followed by Tukey's post-hoc test. The statistical significance was defined as a p-value of less than 0.05 (p < 0.05).

Results

Free radical scavenging activity of the oils

The results of radical scavenging activities of the samples as assessed by their capability to scavenge DPPH or ABTS are shown in Figure 1A and Figure 1B respectively. The DPPH

scavenging activities of the seed oils (figure 1A) increased as the concentration of the extracts increases, reaching as high as $85.057 \pm 0.103\%$ inhibition and $94.829 \pm 0.010\%$ inhibition at the highest concentration of *P. americana* and *C. papaya* respectively. The oils had their lowest DPPH scavenging activity at 10 mg/ml, with *C. papaya* having $93.097 \pm 0.129\%$ and *P. americana* having a value of $82.058 \pm 1.975\%$. At every concentration of the seed oils, *C. papaya* had significantly higher ($p < 0.05$) DPPH scavenging activity than the *P. americana*. The DPPH scavenging activities of the seed oils were significantly lower ($p < 0.05$) than that of the control.

The oils scavenging activity for ABTS (Figure 1B) increased with increasing concentration. At every concentration, *C. papaya* seed oil had ABTS scavenging activity that was significantly higher ($p < 0.05$) than that of *P. americana* seed oil. Again, ABTS scavenging activities of the seed oils were significantly lower ($p < 0.05$) than that of the control. ABTS scavenging activities were highest at 300 mg/ml of the extracts with *C. papaya* having a value of $71.670 \pm 0.423\%$ and *P. americana* having $59.091 \pm 0.317\%$. The least activities were recorded at 100 mg/ml of extracts, with *C. papaya* having a value of $67.442 \pm 0.693\%$ and *P. americana* having a value of $55.391 \pm 1.611\%$.

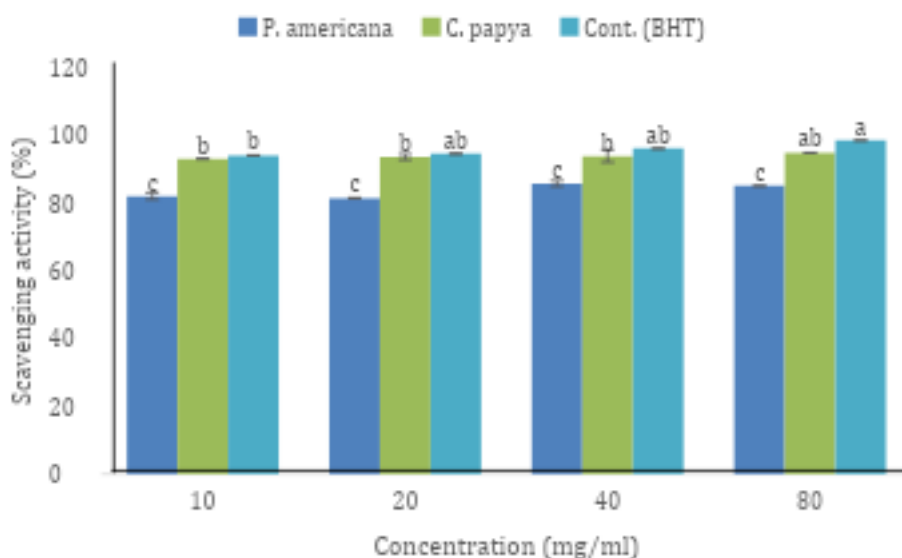


Fig 1A: DPPH scavenging activity of the seed oils of *C. papaya* and *P. americana*. Different alphabets indicate significant difference ($p < 0.05$), where $a > b > c$

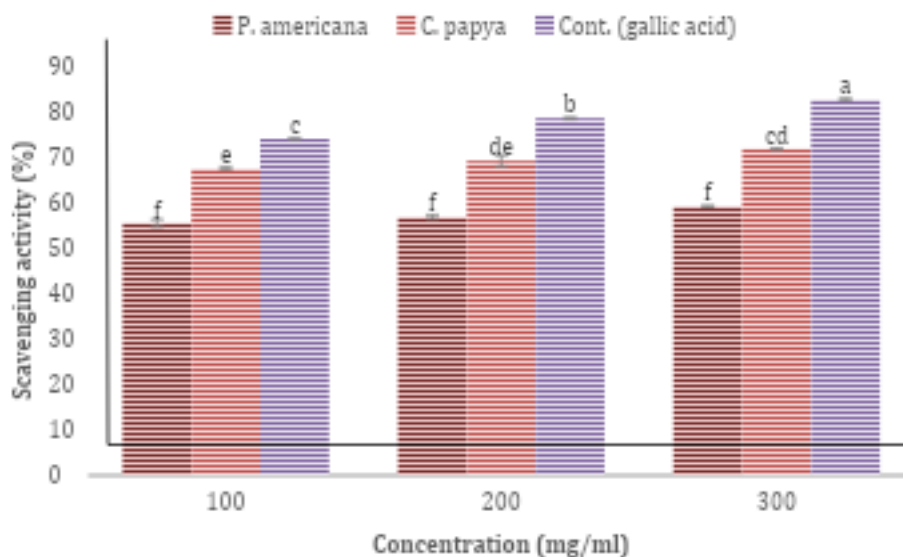


Fig 1B: ABTS scavenging activities of the seed oils of *C. papaya* and *P. americana*. Different alphabets indicate significant difference ($p < 0.05$), where $a > b > c > d > e > f$

SO and H₂O₂ radical scavenging activities of the extracts

Figure 2A and Figure 2B show that the oils can scavenge both superoxide (SO) and hydrogen peroxide (H₂O₂) radicals. The result (Figure 2A) shows that increasing concentration of *P. Americana* seed oil leads to a significant increase ($p < 0.05$) in the superoxide anion radical scavenging activity. However, the SO scavenging activity of *C. papaya* seed oil increases slightly from $72.133 \pm 0.344\%$ to $74.699 \pm 0.402\%$ at 10 mg/ml and 20 mg/ml respectively, while a further increase in concentration to 40 mg/ml and 80 mg/ml, results in a decline in SO scavenging activity. Again, the SO scavenging activity of *C. papaya* was significantly ($p < 0.05$) higher than that of avocado seed oil. *C. papaya* at 10 mg/ml and 40 mg/ml did not show any significant differences ($p > 0.05$) in the SO scavenging activity.

papaya seed oil to 100 mg/ml caused a significant decrease ($p < 0.05$) in the H₂O₂ scavenging activity of the plant. Also, at every concentration, the H₂O₂ scavenging activity of *C. papaya* seed oil was significantly higher ($p < 0.05$) than that of *P. americana*.

Result presented in Figure 2B shows that increasing the concentration of *P. americana* seed oil leads to a significant ($p < 0.05$) increase in the H₂O₂ scavenging activity, with the highest H₂O₂ scavenging activity ($62.156 \pm 0.846\%$) recorded at 100 mg/ml. Again, an increase in concentration of *C. papaya* seed oil from 5 mg/ml to 50 mg/ml, brings about a significant increase ($p < 0.05$) in the H₂O₂ scavenging activity of the plant. However, increasing the concentration of *C.*

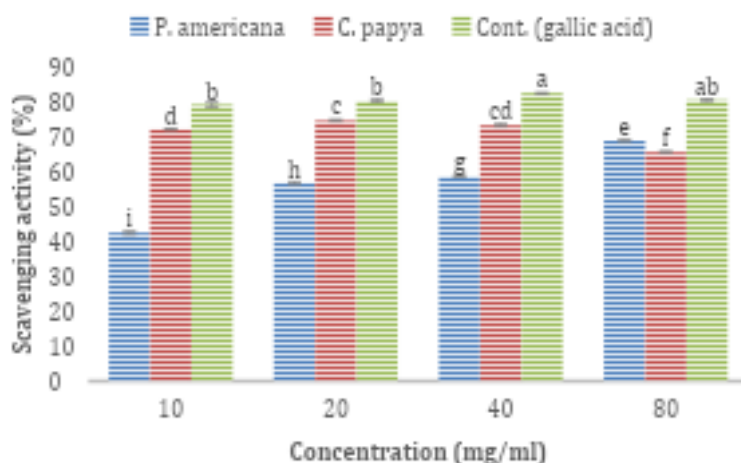


Fig 2A: Superoxide scavenging activity of the seed oils of *C. papaya* and *P. americana*. Different alphabets indicate significant difference ($p < 0.05$), where $a > b > \dots > i$.

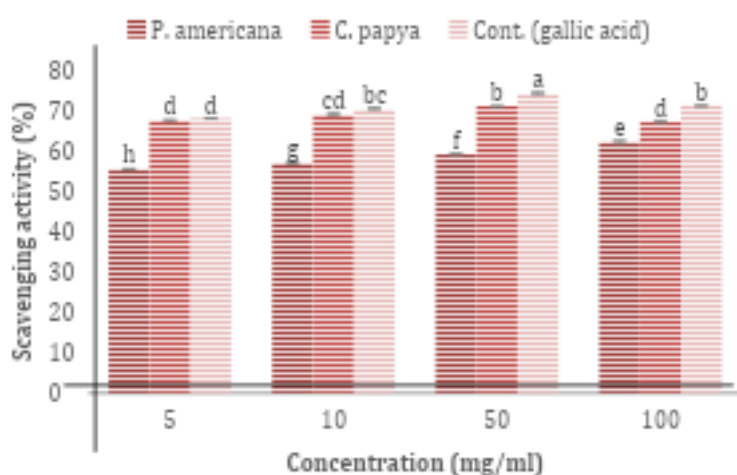


Fig 2B: Hydrogen peroxide scavenging activity of the seed oils of *C. papaya* and *P. americana*. Different alphabets indicate significant difference ($p < 0.05$), where $a > b > \dots > h$

Ferric reducing antioxidant property (FRAP)

The FRAP are shown in Figure 3. The result show that, from 10 mg/ml to 40 mg/ml, the FRAP of *P. americana* significantly ($p < 0.05$) increased, whereas, further increase in concentration to 80 mg/ml does not result in a significant increase ($p > 0.05$) of the FRAP of the extract. Similarly, the FRAP of *P. americana* seed oil was significantly higher ($p < 0.05$) than that of *C. papaya* seed oil. The highest FRAP recorded for the extracts were $89.362 \pm 4.094\%$ and $79.878 \pm 0.264\%$ for *P. americana* (40 mg/ml) and *C. papaya* (80 mg/ml) respectively. Similarly, the least FRAP

recorded in this study were $74.291 \pm 0.532\%$ and $34.198 \pm 1.739\%$ for *P. americana* and *C. papaya* respectively.

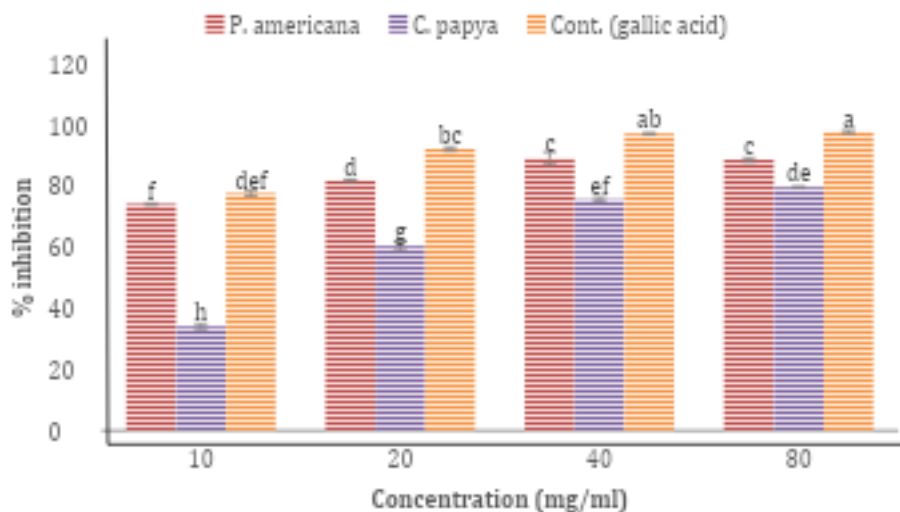


Fig 3: Ferric reducing antioxidant power of the seed extracts of *C. papaya* and *P. americana*. Different alphabets indicate significant difference ($p < 0.05$), where $a > b > \dots > h$

Discussion

It is of utmost importance for there to be a consolidated effort in the search for free radical scavenging plants and their extracts to combat the surge in free radicals which continuously damage many biological cells. Thus, this work evaluated the antioxidant power of avocado seed oil and papaya seed oil by measuring their free radical scavenging and reducing properties. The results of DPPH scavenging activities of the samples are in line with previous works by Kumar *et al.* (2024) and Onyedikachi *et al.* (2024) who opined that percent inhibition of DPPH increases as concentration increases for papaya and avocado seed extracts respectively. The high % inhibitions of the extracts recorded in this work were high compared to that reported by Folasade *et al.* (2016) and Zhou *et al.* (2011) for ethanol extracts of avocado and papaya respectively, further proving the significance of the extracts as a crucial natural free radical scavenger and adding to existing literatures on the antioxidant potentials of the extracts (Dhakad *et al.*, 2018). Again, both the DPPH and the ABTS scavenging activities of papaya were high compared to that of avocado, suggesting that the seed oil of papaya may serve as a better free radical scavenger.

Similarly, SO anion, a major free radical formed from oxidative stress, plays a crucial role in the formation of other ROS such as

H_2O_2 , HO, or O_2 in living systems (Stief, 2003). The observations of the current study highlight the ameliorative effects of the extracts on cellular oxidative damages caused by superoxide anion, as SO radical is reported to cause tissue damage and various diseases through its harmful impact on cellular components (Halliwell and Gutteridge, 1999). Also, the findings of the current work indicate that the oils can act as H_2O_2 scavengers, thus hindering the ability of H_2O_2 to induce injury on other molecules (Sakanaka *et al.*, 2005).

Again, result of FRAP show that increasing the concentration of the oils, results in significant increase in their FRAP power. This is in accordance with the work of Onyedikachi *et al.* (2024). Similarly, the reducing property of avocado seed oil was significantly higher ($p < 0.05$) than the reducing property of papaya seed oil. Thus, the concentration-dependent of DPPH, ABTS, and FRAP observed in the present work suggests that the oils have potentials to scavenge radicals that are harmful in the system.

Conclusion

The study focused on assessing the antioxidant potentials of oils gotten from the seeds of avocado and papaya. The data presented show that the seed oils exhibited strong radical scavenging activities and ferric-reducing abilities in a dose-dependent manner, which was demonstrated with DPPH,

ABTS, SO, H₂O₂, and FRAP antioxidant assays. Results show that the papaya seed oil produced better scavenging effects than the avocado seed oil, whereas the avocado seed oil had stronger ferric-reducing power. Thus, the findings in this study portrays the ability of the oils, especially papaya seed oil, to contribute to ameliorating diseases associated with oxidative stress, developing advanced biomaterials, and acting as essential preservatives in food and pharmaceuticals. Nevertheless, further studies are required to ascertain the phytochemical and nutritional values of these seed oils, to understand the active ingredients responsible for their antioxidant effects.

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