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(Research Article)

Biodrip: A novel approach using Akapulko (*Senna sp.*) leaf bioactive compounds in inhibiting oxidation of used cooking oil

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Abstract

This research explores the potential of bioactive compounds extracted from Akapulko (*Senna sp.*) leaves to inhibit the oxidation of used cooking oil, a significant public health concern due to the frequent reuse of cooking oil in many households. Akapulko leaves, known for their medicinal properties, were identified to contain phenolics, saponins, and terpenoids, which are natural antioxidants. These compounds were extracted using ethanol and tested for their effectiveness through Thin Layer Chromatography (TLC) and peroxide value analysis. The study demonstrated the presence of bioactive compounds in Akapulko extract, confirmed by the formation of distinct color spots on TLC plates. Used cooking oil treated with the extract exhibited a decrease in peroxide value from 3.67 to 3.27 meq/kg, indicating a 10.9% reduction in oxidation. This suggests that Akapulko leaf extracts can act as a natural inhibitor of oxidation, potentially improving the quality and safety of reused cooking oil. This innovation offers a sustainable alternative to synthetic antioxidants, with potential applications in food preservation, public health improvement, and waste reduction. The findings underscore the practicality of utilizing natural antioxidants in everyday settings and open opportunities for commercialization and policy development to promote safer oil reuse practices. This study highlights the promising role of Akapulko leaf bioactive compounds in enhancing the oxidative stability of used cooking oil, addressing both environmental and health challenges.

Keywords: Bioactive; Akapulko (*Senna sp.*); Oxidation; Antioxidants; Peroxide

1. Introduction

Over 278, 000 deaths worldwide have been linked to consumption of oily foods, which are often high in trans fats (World Health Organization, 2024). According to EW Nutrition. (n.d.), oils and fats are prone to rancidity, which not only reduces their nutritional value but also poses significant health risks. In the Philippines, a report by Statista (2024), highlights the 1.11 million metric tons of palm oil consumption out of 79 million metric tons consumed globally. In relation to this, one of the common practices of Filipino households is the repeated use of cooking oil, due to the fact that it lowers the cost of food preparation (Zula & Teferra, 2022). This leads to a growing concern as frequent use of cooking oil at elevated temperatures gradually deteriorates its quality, initiating hydrolysis and oxidation processes (Rusdi et al., 2021). And consuming oxidized compounds from repeated recycling of used cooking oil can pose serious health dangers (Flores & Camacho, 2023).

Natural antioxidants as important bioactive substances with proven use in the food sector (Lourenço et al., 2019). Apart from their application in functional food products, it also served as synthetic counterpart substitutes to improve product stability and prevent oxidation-induced degradation during processing and storage. In addition, Farahmandfar

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et al. (2019) added that plant and herb bioactive substances such as flavonoids, tannins, and hydroxycinnamate esters can offer antioxidant qualities as well as beneficial properties like cardioprotective, antimutagenic, and anticarcinogenic effects. Wherein it can prevent and reduce the oxidation processes of materials that have the potential to produce free radicals (Parcheta et al., 2021).

Senna species, commonly known as Akapulko, is a flowering plant widely recognized for its medicinal qualities particularly, for having antifungal properties. It is locally called “Akapulko” in the Philippines, where it is extensively cultivated for its health benefits. Akapulko is one of the ten medicinal plants recommended by the Department of Health (DOH), validated for its safety and efficacy in treating various ailments (Quiñones, 2023). This plant, also known as the Christmas candle or candle tree (Adebayo et al., 2023), is not only valued for its medicinal use but also holds potential for applications in other sectors, such as food preservation.

According to the study of Rahmawati et al. (2022), Senna leaves contain bioactive compounds that act as antifungals and these active compounds are alkaloids, flavonoids, saponins, tannins, triterpenoids, steroids, and glycosides. Alshehri et al. (2022) also noted that Senna plants are powerful antioxidants and antimicrobials that have a variety of positive health impacts. Given the bioactive compounds and antioxidant properties of Akapulko (*Senna sp*) leaf, utilizing its extracts can effectively inhibit the oxidation of used palm cooking oil, thereby reducing the health risks associated with the consumption of degraded oil. By incorporating Akapulko extracts into oil preservation, this novel approach not only offers a practical method to improve public health but also, it offers a sustainable solution to reduce food waste.

Objectives of the Study

This research is conducted in order to extract the bioactive compounds from Akapulko (*Senna sp.*) leaf and analyze its efficacy in inhibiting degradation of used palm cooking oil. Specifically, it aimed to:

- Determine the qualitative intensity of bio-active compounds present in Akapulko (*Senna sp.*) leaf in terms of phenolic, saponins, and terpenoids.
- Measure the Peroxide value of the used oil via pre and post Akapulko (*Senna sp.*) leaf extract treatment.
- Determine the descriptive relationship among the peroxide values of the set-ups.

1.1. Significance of the Study

The bioactive compound extracts from Akapulko (*Senna sp.*) leaf will help inhibit the oxidation of used palm cooking oil which is significant for the following reasons:

- **Reduced Food Waste:** By allowing palm cooking oil to be used safely for longer periods, this reduces the need to discard the oil after a single use. Thus, aligning with Sustainable Development Goal 12 (target 12.3).
- **Improved Oil Quality:** Treatment of used palm cooking oil with the bioactive compounds of Akapulko (*Senna sp.*) leaf will help preserve the sensory qualities (color, smell, taste) of the oil over time.
- **Reduction of Harmful Compounds:** The application of these bioactive compounds will help lessen the accumulation of harmful compounds from the repeated use of palm cooking oil through reduced peroxide values indicating decreased formation of peroxides, which are primary oxidation products.
- **Natural and Safe Alternative:** The bioactive compounds extracted from Akapulko leaf will provide an alternative to synthetic antioxidants, potentially reducing reliance on chemical preservatives.
- **Practical Applications:** The findings in this study could suggest that this method is practical for widespread use, potentially leading to commercialization opportunities that could be marketed to households or the food industry. As well as policy implications wherein, it can help inform guidelines and recommendations for the safe reuse of cooking oils particularly, in regions where oil recycling is common.

1.2. Scope and Limitations of the Study

This study centers on extracting Akapulko (*Senna sp.*) leaf bioactive compounds to inhibit the oxidation of used cooking oil particularly, palm oil. Wherein, it will measure the effectiveness of the Akapulko liquid extracts through determining the peroxide value of the treatments and the relationship between the peroxide value of the treated and untreated used cooking oil.

Furthermore, this study will not include or measure other variables that might affect the results of the treatment, such as the heating temperature of the oil, the amount of time the oil was heated, and the timing in which the Akapulko extracts were added in the cooking oil either before or after. Also, this research will not quantify the amount of bioactive compounds present in the Akapulko (*Senna sp*) leaf, limiting its focus to phenols, saponins, and terpenoids.

2. Material and methods

2.1. Materials

The laboratory equipment used to conduct the procedures necessary for this study includes an Erlenmeyer flask, graduated cylinder, funnel, beaker, glass stirring rod, dropper, wire gauze, utility clamp, ring stand, thermometer, iron ring, alcohol lamp, electronic balance, spatula, tripod, storage bottles, and vials. Additionally, solar dryer and blender were utilized in drying and grinding. Finally, 100-mL of Palm cooking oil and 100 g of Akapulko leaves were prepared.

2.2. Methods

2.2.1. Collection and Preparation of Akapulko Leaf Extracts (Adebayao et al., 2021; Muruhan et al.,2013)

Akapulko (*Senna sp.*) leaves were gathered from the local areas within Barangay 23, Gingoog City and were shade-dried for 2-3 days. Afterwards, the dried leaves were ground into a fine powder using a blender. Wherein, one hundred grams of dry powder was suspended in 400 mL of ethanol for 72 hours.

2.2.2. Ethanol Evaporation on Bioactive Compounds (Bennour et al., 2019)

Subsequently, the evaporation method was undertaken at room temperature to lessen the ethanol contents in the extract. This approach was adapted by using a hot water bath set at 40°C for 2 hours, to expedite the evaporation process.

2.2.3. Thin Layer Chromatography Assay (Guevarra, 2005)

Method for TLC followed Guevarra, B. (2005). A Guidebook to Plant Screening: Phytochemical and Biological Revised Ed. University of Santo Tomas Publishing House. Manila.2. Chapter-IV-Qualitative Phytochemical Investigation. Test methods for Terpenoids and Phenols were Ethyl acetate: acetic acid: water: formic acid (100:11:11:26) developing solvent system and 0.5% vanillin-sulfuric acid as derivatizing agent. Chloroform: methanol (30:5) developing solvent system and derivatized with 1% Anisaldehyde- sulfuric acid reagent was used for test for Saponins.

2.2.4. Thermal Heating and Treatment of Oil Samples (Tuangco et al., 2021)

The 100-mL palm cooking oil was heated between 100°C and 200°C, since heating oils at elevated temperatures eventually initiates the oxidation process (Ganesan et al., 2019). Subsequently, it was cooled down through submerging the beaker containing the PCO sample in a container filled with water. When the temperature of the cooking oil was already below 40°C, the Akapulko leaf extracts were then added to each PCO sample. Wherein in every 25 mL solution comprising PCO samples, 0.5% is the Akapulko leaf extract, which served as the limitation in the amount of additives safe for consumption (Tuangco et al., 2021). Lastly, each solution was mixed and kept in a storage bottle for the laboratory analysis.

2.2.5. Peroxide Value (Francisca et al., 2023)

Analysis from AOAC International, 21st ed., 2019 of Titrimetric method was used for the determination of the peroxide value. A ~5.0 g of each sample in a 3:2 acetic acid-chloroform solution, adding saturated potassium iodide, shaking, and then titrating with 0.1 M sodium thiosulfate after adding water and starch solution. The peroxide value (PV) was calculated using a formula that accounts for the titrant volumes of the sample and blank, sodium thiosulfate concentration, and the sample's mass (Francisca et al., 2023).

2.2.6. Data Analysis

Descriptive analysis was used to interpret the peroxide values of used cooking oil before and after treatment with Akapulko (*Senna sp.*) extract. Since only one measurement was available for each condition, statistical testing was not feasible. Descriptive analysis provides a straightforward approach to assess the percentage change in peroxide values, allowing for an initial understanding of the potential antioxidative effect of the Akapulko extract.

3. Results and discussion

3.1. Thin Layer Chromatography

Thin-layer chromatography (TLC) is an easy and economical method that is widely employed in the analysis of natural products. It is ideal for the chemical and biological assessment of herbal products and is valuable for tracking the bioguided isolation of active compounds (Urbain & Simões-Pires, 2020).

Table 1 Qualitative results thin layer chromatography

Parameters	Sample	Results	Standard Reference	Test Methods
Qualitative Test for Terpenoids	Acapulco	(+) Formation of a violet/blue/reddish spot	Formation of a red/violet spot upon derivatizing with 0.5% vanillin-sulfuric acid	Ethyl acetate: acetic acid: water: formic acid (100:11:11:26) developing solvent system and 0.5% vanillin-sulfuric acid as derivatizing agent
Qualitative Test for Phenolics	Acapulco	(+) Formation of a visible blue spot	Formation of a visible blue spot upon heating at 110°C after derivatizing with 2% ethanolic FeCl3	Ethyl acetate: formic acid: acetic acid: water(100:11:11:26) developing solvent system and 2% ethanolic FeCl3
Qualitative Test for Saponins	Acapulco	(+) Formation of a violet spot	Formation of blue/red/violet spots upon heating at 110°C after derivatizing with 1% Anisaldehyde-sulfuric acid reagent	Chloroform: methanol (30:5) developing solvent system and derivatized with 1% Anisaldehyde-sulfuric acid reagent

Table 1 shows the Qualitative results of Thin Layer Chromatography for Terpenoids, Phenolics, and Saponins which are bioactive compounds present in Akapulko (*Senna sp.*) leaf. For the first parameter, formation of a violet/blue/reddish spot confirmed the presence of Terpenoids when derivatized using 0.5% vanillin-sulfuric acid. In terms of the second parameter, Phenolics was also detected through the formation of a visible blue spot when heated at 110 °C following derivatization with 2% ethanolic FeCl3. And for the third parameter, the presence of Saponins was displayed through the formation of a violet spot upon heating at 110°C following derivatizations with 1% Anisaldehydesulfuric acid reagent.

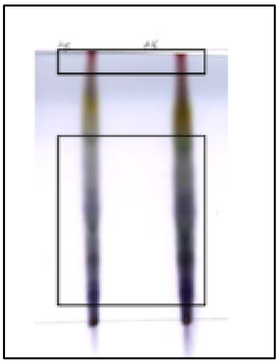

Figure 1 Image of the TLC profile of Acapulco showing visible red/violet/blue spots upon derivatizing with 0.5% vanillin-sulfuric acid

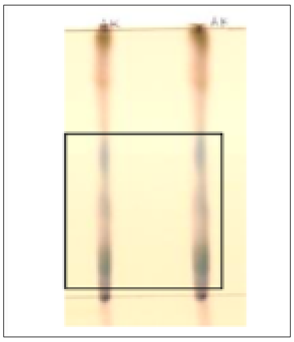

Figure 2 Image of the TLC profile of Acapulco showing bluish-violet spot upon derivatizing with 2% ethanolic FeCl3.

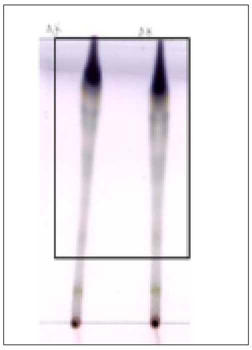

Figure 3 Image of the TLC profile of Acapulco showing violet spot upon heating after derivatizing with 1% Anisaldehyde-sulfuric acid.

Important structural insights that are overlooked if only the final color is taken into account are contained in the color information, which is represented as RGB values, and the complete color production process, including starting, intermediate, and final colors. This approach makes it possible to more effectively identify bioactive chemicals by utilizing TLC as a high-throughput, portable, and easily accessible choice for extensive screening and initial investigation of natural products (Xu & Liu, 2021).

3.2. Peroxide Value

Peroxide Value serves as a crucial measure for tracking the oxidation of oil during its storage. It provides insights into the early stages of fat degradation and is a vital metric utilized in food safety and quality assessment (Cocan et al., 2022; Zhang et al., 2021).

Table 2 Peroxide Value Results

Sample	Results (Peroxide Value, meq/kg)	Test Method
Used Oil (pre-treatment)	3.67	Titrimetric
Used Oil (post-treatment)	3.27	Titrimetric

3.3. Thin Layer Chromatography

According to Wutsqa et al. (2021), red-violet stain signals the presence of terpenoid compounds. Wherein, the violet hue is often associated with monoterpenes and certain triterpenes, which are the building blocks of terpenoids (Ninkuu et al., 2021). In Figure 1, the colors produced after derivatization are associated with the chemical nature of the compound (Agatonovic-Kustrin et al., 2019). In Figure 2, the violet spots detected indicates phenolic molecules which strongly signifies the content of phenols as a bioactive compound (Agatonovic-Kustrin et al., 2019). While the bluesh spot is the result of the interaction between the phenols and the ethanolic FeCl3. As shown in Figure 3, the image greatly aligns with Qin et al. (2023) emphasizing that violet spot is an indicator of steroid and triterpenoid, which are major classes of saponins. This implies the existence of saponins as one of the bioactive compounds of Akapulko (*Senna sp.*) leaf.

As cited in Rahman et al. (2023), Akapulko (*Senna sp.*) leaf contains bioactive compounds that contribute to its antioxidant properties. Specifically, saponins, terpenoids, and phenolics that was identified through the formation of visible spots as shown in the images.

3.4. Peroxide Value

Bioactive compounds involving antioxidants play a crucial role in decreasing oxidation rate by inhibiting free radicals (Yu et al., 2021). Walayat et al. (2023) further added that oxidation may be inhibited by the increased bioactive components incorporated in used cooking oil.

In the study of Gutiérrez-del-Río et al., 2021, terpenoids and phenols are natural antioxidants that play crucial roles in preventing oil oxidation. Terpenoids, such as carotenoids, neutralize reactive oxygen species like singlet oxygen, while phenols, including flavonoids, stabilize free radicals and chelate prooxidant metals, thereby reducing lipid peroxidation. Such findings line with the results of the research. Together, these compounds synergistically preserve the sensory and nutritional quality of oils, making them effective natural alternatives to synthetic antioxidants).

Saponins exhibit significant effects in mitigating oxidation processes, as highlighted in the study of Liu et al. (2022). These naturally occurring substances' distinct structure, which consists of hydrophilic glycosides and hydrophobic triterpenoid ligands, gives them antioxidant qualities. Saponins' dual nature enables them to work well with lipid and protein interfacial systems to form barriers that prevent oxidative processes. Lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS), which are indicators of primary and secondary lipid oxidation, respectively, can be decreased by saponins.

3.5. Determine the descriptive relationship among the peroxide values of the set-ups.

Based on the results of the Peroxide Values presented in Table 2, the value for the used cooking oil without the treatment of Akapulko leaf extracts is 3.67 meq/kg, while the peroxide value of the used cooking oil with Akapulko leaf extracts treatment is 3.27 meq/kg. This shows that there is a decrease of about 10.9% in the peroxide value, calculated as follows:

$$Percentage\ Reduction\ = \frac{3.67\ meq/kg - 3.27\ meq/kg}{3.67\ meq/kg} \times 100 = 10.9\%$$

With a percentage reduction of 10.9%, this suggests the effectivity of the bioactive compounds of Akapulko (*Senna sp.*) as natural inhibition of oxidation in used cooking oil. As noted by Buthelezi et al. (2019), oils with relatively low peroxide values (< 10 meq O2/kg) show that the products are stable against oxidation and oils with high peroxide values (> 10 meq O2/kg) are considered unstable and easily become rancid.

4. Conclusion

The findings displayed a positive qualitative intensity of bio-active compounds present in Akapulko (*Senna sp.*) leaf extract in terms of phenolic, saponins, and terpenoids through Thin Layer Chromatography. Various indicators in terms of color confirmed the availability of bioactive compounds in the Akapulko (*Senna sp.*) liquid extract. A visible formation of a red/violet/blue, bluishviolet, and violet spot indicated strong presence of terpenoids, phenolics, and saponins respectively. Used cooking oil as medium pre to post treatment resulted a 0.40 meq/kg difference giving 10.9% inhibition of oxidation. The peroxide value suggests the effectivity of the bio-active compounds present in the Akapulko (*Senna sp.*) leaf extract as inhibitors for oxidation on used cooking oil.

The outcome underscores the practical and sustainable benefits of utilizing the bioactive compounds containing natural antioxidants from Akapulko leaf extracts as an alternative to synthetic preservatives. This innovation not only contributes to healthier consumption by mitigating health risks associated with rancid oils but also supports environmental goals by reducing food waste. The study opens avenues for broader applications, including commercialization in the food industry and integration into public policies for safer oil recycling practices.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors, including the corresponding authors, confirm that they have no conflicts of interest pertaining to the publication of this paper.

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