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RESEARCH ARTICLE

EVALUATION OF ATILI (*Canarium schweinfurthii*) FRUIT OIL FROM DIFFERENT EXTRACTION METHODS FOR POTENTIAL USE AS A NATURAL BIO-PRESERVATIVE

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ABSTRACT

This study evaluated the quality indices and antimicrobial activity of oils extracted from *Canarium schweinfurthii* (Atili fruit) using four methods: soxhlet, conventional extraction (with modification), cold solvent, and cold press oil extraction methods. The soxhlet method produced the highest oil yield (44.72%) and the lowest peroxide value (77.27 meq/kg), indicating greater oxidative stability. The conventional method yielded oil with the lowest acid value (38.15 mg KOH/g) and free fatty acid content (19.17 mg KOH/g). Soxhlet extracted oil had the least density (0.92 g/mL), while cold solvent-extracted oil had the lowest specific gravity (0.94). The antimicrobial properties of the extracted oils were tested against food spoilage and pathogenic microorganisms, including *Aspergillus flavus, Penicillium sp.*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus,* and *Saccharomyces sp.* using agar-well diffusion and micro-broth dilution methods. The oils demonstrated broad-spectrum antimicrobial activity, with *P. aeruginosa* showing the least susceptibility and spoilage yeast being the most susceptible. Antibacterial activity was achieved at an average oil concentration of 6.25%, while antifungal effectiveness was sustained at concentrations above 12.50%. These findings highlight the potential of *C. schweinfurthii* oil, particularly soxhlet-extracted oil, as a natural bio-preservative for fruits and vegetables, providing an environmentally friendly alternative to synthetic preservatives for post-harvest loss reduction.

Keywords: Antibacterial, antimicrobial, Atili, *Canarium schweinfurthii*, oil quality

INTRODUCTION

Indigenous fruits play an essential role in sustaining the livelihoods of rural communities across Nigeria, particularly in arid regions. Ethnobotanical studies have underscored the significance of *Canarium schweinfurthii* (commonly known as African black olive, African elemi, or bush candle) due to its medicinal applications and edible fruit. This perennial tree, a member of the Burseraceae family, thrives in West and Central Africa, as well as parts of Northern and Eastern Nigeria. It is locally known as 'Ube mgba' (Igbo), 'Atili' (Hausa), or 'Origbo' (Yoruba), with its pulp containing 40–50% oil, highly valued for traditional uses (Nyam *et al*., 2013; Anyalogbu *et al*., 2014; Wahab *et al*., 2015; Mohammed *et al*., 2022).

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Traditional oil extraction methods, such as fermentation, are still prevalent in regions where *C. schweinfurthii* is abundant. These methods involve processes like pre-warming and partial drying. This is then followed by mashing the fruit and allowing to ferment, and then decanting, and sieving. Such techniques are believed to enhance flavor and facilitate the separation of oil from other components like carbohydrates, proteins, and water phases (Nyam *et al*., 2013; Agu *et al*., 2008). Despite the increasing demand for *C. schweinfurthii* oil, limited attention has been given to optimizing extraction techniques, particularly

in developing countries. Modern methods could potentially improve oil yield and quality, which remains a key focus of this research.

In addition to its traditional uses, *C. schweinfurthii* oil offers potential as a natural preservative. Synthetic preservatives such as benzoic acids, benzoates, sulfites, sorbates etc. are widely used in food and agriculture to mitigate post-harvest losses in tropical regions like Nigeria (FAO, 2019; Omodamiro *et al*., 2020). However, concerns about health and environmental impacts including carcinogenicity (Bergman *et al*., 2012), allergic reactions (Vally and Misso, 2012), disruptions to microbiomes (Rodriguez, 2019), ecological harm (Guerra-Rosas *et al*., 2020), and the emergence of resistant microorganisms have driven the search for safer alternatives (Kumar and Singhal, 2020). Plant-based oils, known for their bioactive compounds such as phenols, terpenes, and alkaloids, are increasingly recognized for their antimicrobial potential. These oils have shown promise in preventing the growth of microorganisms and elongating the shelf-life of agricultural produce (Santos and Silva, 2020; Daferera *et al*., 2021; Viegas *et al*., 2022).

Given the culinary, cultural, and potential health benefits of *C. schweinfurthii* fruit oil (Ogunwande *et al*. 2003; Nguta *et al*., 2010), this study evaluates the quality indices of *C. schweinfurthii* oil extracted using four different methods; soxhlet, conventional, cold solvent, and cold press and examines its antimicrobial activity against selected microbial pathogens. The findings aim to assess the potential of this tropical oil as a natural bio-preservative to reduce post-harvest losses and promote sustainable agricultural practices.

MATERIALS AND METHODS

Sample collection and preparation

C. schweinfurthii fruits were procured from Orange Market in Maraba, Nasarawa State. They were sorted and placed in an already cleaned bowl with hot water (65 °C) and left for 5 minutes to soften the tissues. The fruits were then deseeded and the pulp was collected and mashed thoroughly using mortar and pestle. This was then oven-dried for 1 hr using a hot air oven at 60 °C and kept for further use (Agu *et al*., 2008).

Sample extraction

Four (4) different extraction methods were employed to obtain oil from the prepared sample. These are soxhlet extraction, conventional solvent extraction, cold solvent extraction, and cold (mechanical) extraction.

The soxhlet extraction method was carried out according to the procedure described by the Association of Official Analytical Chemists (AOAC, 2019). Fifty gramme (50 g) of the dried pulp was placed in a cellulose thimble and placed in the soxhlet apparatus for continuous extraction using n-hexane as the solvent for 6 hours. After extraction, the solvent was recovered and the remaining oil was dried in the oven at 105 °C for 30 minutes.

The conventional solvent extraction method was done following the method reported by Nyam *et al*. (2013) with modification. About 200 g of the dried pulp was mixed with sufficient amount of hot water. The mixture was subjected to low heat and allowed to stand for 4 hours, after which the oil formed at the upper layer with the pulp was scooped and sieved using muslin cloth to separate the oil. The resulting solution was further dried in the oven at 105 °C to remove excess water.

The cold solvent extraction method followed the procedure of Reyes-Jurado *et al*. (2014). About 200 g of the dried pulp was soaked in n -hexane at ambient temperature (28 ± 2 °C) in a well stoppered 500 mL conical flask and kept for 48 hours with occasional shaking. After 48 hours, the solution was filtered using a muslin cloth. The residue was further washed in little amount of n-hexane and re-filtered. All the filtrates were gathered together and subjected to rotary evaporation to remove the solvent from the oil, which was later dried in an oven to remove excess solvent.

The cold press (mechanical) extraction method, as described by Abah and Egwari (2011), involved further grinding the dried

pulp using an electric blender and warm water was briefly sprinkled on the ground pulp before being manually pressed using a clean muslin cloth to collect the oil.

All extracted oil samples were weighed individually and kept in separate amber glass bottles for further use.

Oil quality analyses

The oil yield in percentage was estimated as described by AOAC (2000). Density and specific gravity of each oil was determined using the Pycnometric method of AOAC (2019). Refractive indices η/D30 (RI), were determined from an Abbe refractometer at 30±0.1 °C according to AOAC (2019). Saponification value, peroxide value, acid value and free fatty acid (FFA) were all analyzed via titrimetric methods as described by AOAC (2019). Iodine Value was estimated using a mathematical relationship between refractive index and iodine value as described by Perkins and Zimmerman (1995) and reported by Onyegbula *et al.* (2020).

Antimicrobial effectiveness

With 0.5 McFarland standardized (1.5 x 10^7 CFU/ml) final bacterial inoculum and 1.0 x 10^6 CFU/mL haemocytometer-measured fungal inoculum, the antimicrobial effectiveness testing of extracted oils using the agar-well diffusion method were conducted on Mueller Hinton Agar as described by AOAC (2019) with slight modifications. Minimum Inhibitory Concentration (MIC) and Minimum Biocidal Concentration (MBC) were assayed in modified Micro-broth dilution method in Bacteriological Peptone (HKM HAM005, India) and Mycological Peptone (OXOID LP0040B, UK) appropriately (Calvo *et al*., 2011) for the fungi, *Aspergillus flavus,* and *Penicillium sp.,* the bacteria, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus,* and the yeast, *Saccharomyces sp*. Experimental sets were in triplicates incubated overnight at 35±2 °C and 30±5 °C (for bacteria and fungi respectively) for 24±2 hrs. Zones of inhibitions were measured in millimeter; clarity/turbidity of tubes assessed visually and biocidal functions confirmed with standard pour-plating on appropriate culture medium.

Statistical analyses

Collected data, in triplicate, was subjected to ordinary analysis of variance (ANOVA) and tested for significant difference by Tukey's Multiple Comparisons ($p<0.05$) using GraphPad Prism 8.0.2 software.

RESULTS AND DISCUSSION

Quality properties of *C. schweinfurthii* oils extracted by different methods

The result revealed that soxhlet extraction method is effective in maximizing oil yield, achieving a significant yield of 44.72%, the highest recorded in this study (Figure 1). Cold solvent extraction method, in contrast, yielded 14.83%, which is significantly lower than that of soxhlet, but superior to the traditional cold pressing method. Both cold press and conventional extraction methods exhibited lower yields with reported values of 8.63% and 6.94% respectively.

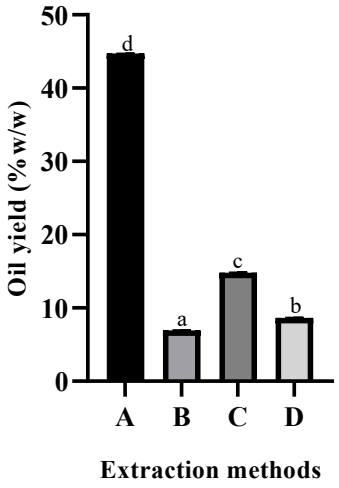

Figure 1: Oil yield (%) of *C. schweinfurthii* oils obtained by soxhlet, conventional, cold-solvent, and cold-press methods

Data represent mean ± SE of triplicate readings. A: Soxhlet extraction method, B: Conventional extraction method, C: Cold extraction method, D: Cold press extraction method

The observed variability in oil yields corroborates the findings of Adebayo *et al*. (2015) on different extraction methods exhibiting different efficiencies based on the

techniques used for the extraction of kariya seed oil. Soxhlet and cold solvent extraction methods have been noted in literatures to generally have a significantly high extraction capabilities, thus highlighting the limitations of the traditional cold press and conventional methods of extraction when compared (Juhaimi *et al*., 2018; Omeje *et al*., 2022). Although the cold press extraction yields lower than the solvent-based methods, it is often favoured for its minimal processing and preservation of bioactive compounds, antioxidant activities and phenolic contents relative to the solvent-based extraction methods (Juhaimi *et al*., 2021).

Density and specific gravity are essential physicochemical properties that reflect the molecular compactness, and compositional integrity of edible oils, which can aid in identifying impurities or adulteration (Yahaya *et al*., 2012). While specific gravity compares the density of an oil to that of water, the density explains the mass of an oil in relation to its volume. In this study, the density of the *C. schweinfurthii* oils ranged from 0.92 to 0.93 g/mL, and specific gravity from 0.94 to 0.96 with soxhlet extraction method having the least density and cold solvent extraction showing the lowest specific gravity (Figure 2). The density and specific gravity values obtained for *C. schweinfurthii* oils are consistent with those reported for high-quality tropical fruit oils (Ayodele *et al*., 2022) which suggest minimal adulteration and considerable purity. According to Ichu and Nwakanma (2019), edible and purer oils typically have lower density than water, such as those reported in this study. Oils with optimal values are more stable, forming no phase separation over time, making them essential for application in food preservation (Olagunju *et al*., 2022).

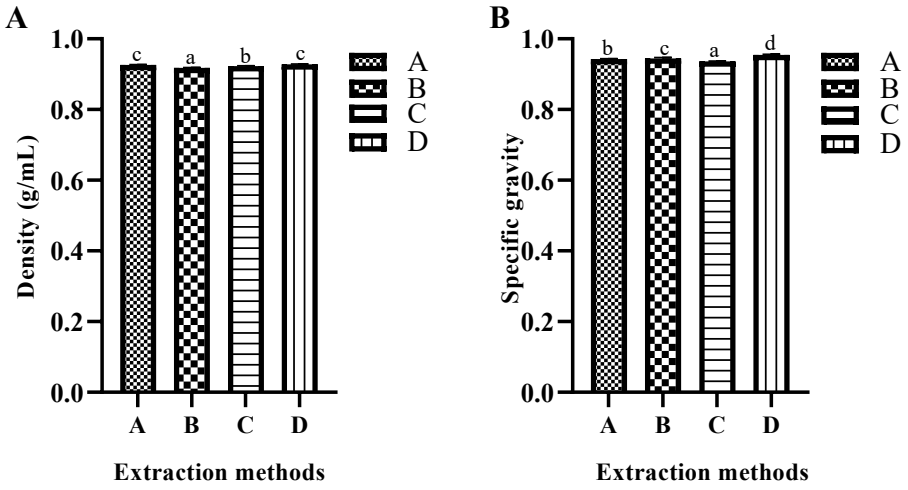

Figure 2: Density (g/mL) (A) and specific gravity (B) of *C. schweinfurthii* oil extracted using soxhlet, conventional, cold solvent, and cold press methods

Data represent mean ± SE of triplicate readings. A: Soxhlet extraction method, B: Conventional extraction method, C: Cold extraction method, D: Cold press extraction method

The saponification value of oils is explained as the amount in miligrams of potassium hydroxide required to saponify 1 g of oil (Table 1). The saponification value of the oils in this study ranged from 242.69 to 263.91 mg KOH/g, which are relatively high when compared to 213 mg KOH/g neem seed oil and 253 mg KOH/g in coconut oil as reported by Abdulhamid *et al*. (2014). A high saponification value indicates a high proportion of short-and medium-chain fatty acids (MCFAs) composition in the oil (Adeyeye *et al*., 2023; Ogunniyi *et al*., 2022). These MCFAs have been reported for their significant antimicrobial properties which helps to inhibit the growth of food spoilage

and pathogenic microorganisms thereby preserving and improving the shelf-life of the food (Afolayan *et al*., 2023; Bai *et al*., 2021). In addition, oils with high saponification values incline to have pronounced emulsifying properties, which enhances their ability to form stable emulsions, beneficial for use as coating or spray on fresh produce for preservation purposes (Ayodele *et al*., 2022; Bai *et al*., 2021). These underscore the suitability and potential use of *C. schweinfurthii* for the post-harvest management of fresh fruits as well as other agricultural commodities.

Table 1: Properties of *C. schweinfurthii* oils extracted by different methods

Oil quality indices	A	B	C	D
Saponification value (mg KOH/g)	242.69±0.05^{a}	259.37±0.03^{c}	250.37±0.02^{b}	263.91±0.07^{d}
Refractive index	1.47±0.00^{a}	1.47±0.00^{a}	1.47±0.00^{a}	1.47±0.00^{a}
Acid value (mg KOH/g)	47.47±0.05^{d}	38.15±0.08^{a}	45.23±0.07^{b}	47.12±0.05^{c}
FFA (mg KOH/g)	23.86±0.02^{d}	19.17±0.04^{a}	22.73±0.04^{b}	23.68±0.03^{c}
Peroxide (meq/kg)	77.27±0.04^{a}	362.94±0.04^{c}	191.22±0.24^{b}	415.65±0.13^{d}
Iodine Value (gI_2/100g)	79.75±0.29^{c}	76.32±0.29^{a}	77.46±0.29^{b}	82.05±0.50^{d}

Values are mean±Standard error (SE) of triplicate measurements. Means with unshared superscript in the same column are significantly different at 95% confidence level ($p<0.05$). A: soxhlet extraction method, B: conventional extraction method, C: cold solvent extraction method and D: cold press extraction method.

The refractive index of oil, which measures how light is bent as it passes through the oil, provides valuable insights into its purity, concentration, and degree of unsaturation (Adeyeye *et al*., 2023). A higher refractive index typically indicates greater levels of adulteration or impurities adulteration (Vishakh and Rageena, 2018). In this study, the refractive index of the oils were 1.47, although higher than the average refractive index of edible coconutoil (1.445), falls within the Codex Alimentarius standards for the refractive index of mustard seed, sesame, soya, and olive seed oils, as reported by Ichu and Nwakanma (2019).

The acid value and FFA content of the oils analyzed in this study exhibited significant differences ($p<0.05$) among the extraction methods, with the conventional extracted oil showing the least values (38.15 and 19.17 mg KOH/g for acid value and FFA respectively). These findings significantly surpass recommended value of 0.6 to 4.0 mg KOH/g for processed and cold-pressed oils, as specified by CODEX 2005. Acid value represents the concentration of FFA in an oil, while FFA content reflects the extent of triglyceride breakdown. Elevated values for these indices, as reported in this study, indicate hydrolysis or oxidation of triglycerides, which can result in rancidity, undesirable flavors, reduced stability, and a shorter shelf life (Aluyor *et al*., 2009). Thus, to improve the shelf-life, sensory quality and food applications where flavor and stability is important, the extracted *C. schweinfurthii* oils need to be refined (Ayodele *et al*., 2022). While the high acid values limit direct consumption of these oils, they may enhance the effectiveness of the oils as a natural preservative for post-harvest management purposes, as FFAs inhibit microbial growth (Afolayan *et al*., 2023).

Peroxide value of oil is an important oil quality index that indicates lipid oxidation and estimates the concentration of peroxides and hydroperoxides formed during the early stages of oxidative rancidity (Olagunju *et al*., 2022). According to Codex Alimentarius, 10-20 meq/kg is the recommended maximum limit for peroxide value of edible oils. The observed peroxide values in this study revealed considerable variation among the oils, and all the values exceed the maximum limit, with soxhlet-extracted oil recording the least (77.27 meq/kg). This suggests that the oils are highly prone to oxidative rancidity and could be as a result of oxidative stress during extraction, exposure to air, high degree of unsaturation or lack of/low concentration of antioxidants (Olagunju *et al*., 2022; Ogunniyi *et al*., 2022) which could potentially

be enhanced through antioxidant fortification (Kyari, 2008). Although the recorded high peroxide value of the extracted oils can compromise its safety, flavor and nutritional quality, it can positively imparts the antimicrobial activity as reactive oxygen species can be generated from peroxide breakdown, which cause a damage to microbial cells (Bai *et al*., 2021). This implies that the extracted oils are unsuitable for consumption but contributes to its potential use as bio-preservative for surface treatment of fruits and vegetables.

Iodine value, a measure of oil unsaturation, directly correlates with the risk of rancidity; the higher the iodine value, the greater the degree of unsaturation and the susceptibility to oxidative degradation (Aluyor *et al*., 2009). The oil extracted using the conventional method recorded the lowest iodine value (76.32 gI_2/100g), while others aligns with the iodine values reported for olive oil, typically ranging from 79 to 94 gI_2/100g (Shahidi, 2020; Ayodele *et al*., 2022). Iodine values of oil can contribute significantly to its preservative role as an antimicrobial agent through the constituent unsaturated fatty acids causing a disruption to the microbial cell membranes and inhibiting the growth of pathogens (Afolayan *et al*., 2023).

Antimicrobial effectiveness of *C. schweinfurthii* oils extracted by different methods

The antimicrobial efficacy of the extracted oils exhibited broad-spectrum activity against selected bacterial and fungal food spoilage organisms. Among the tested bacteria, *E. coli* was identified as the most susceptible pathogen, as it was well inhibited by all the oils, with soxhlet extracted oil recording the most inhibition 11.0 mm. *Pseudomonas aeruginosa* showed relatively high resistance to the oils, with total resistance recorded for conventional and cold press extracted oils (Table 2). This can be attributed to its known efflux pump mechanism and robust cell wall structure as reported (Afolayan *et al*., 2023). The fungal species, and yeast were considerably inhibited by all the extracted oils except for cold press extracted oil which was totally resisted by *A. flavus* and *Penicillium sp.* These findings are consistent with previous studies (Ogunwande *et al*., 2003; Ezeonu and Chidume 2008; Oyemitan *et al*., 2010), which reported antimicrobial activity in essential oils and other parts of *C. schweinfurthii*. This underscores the potential of this underutilized fruit as a natural alternative to synthetic food preservatives.

Table 2: Measured inhibitions exhibited by *C. schweinfurthii* oils (Diameter of zones in mm)

Test microbes	A	B	C	D
Escherichia coli	11.00 ± 2.40^{d}	7.00 ± 0.33^{b}	6.00 ± 0.67^{a}	8.00 ± 1.20^{c}
Klebsiella pneumoniae	6.70 ± 0.67^{c}	4.70 ± 0.67^{b}	7.00 ± 0.58^{c}	3.00 ± 1.00^{a}
Pseudomonas aeruginosa	1.70 ± 0.67^{a}	R	3.00 ± 0.33^{b}	R
Staphylococcus aureus	5.00 ± 0.88^{b}	1.70 ± 0.33^{a}	5.70 ± 0.33^{c}	5.00 ± 0.67^{b}
Aspergillus flavus	4.70 ± 0.88^{b}	2.70 ± 0.33^{a}	5.00 ± 0.33^{b}	R
Penicillium sp.	3.00 ± 0.33^{b}	1.00 ± 0.00^{a}	4.00 ± 0.68^{c}	R
Saccharomyces sp.	4.70 ± 0.67^{b}	3.00 ± 0.33^{a}	5.70 ± 0.67^{c}	5.00 ± 0.58^{b}

Values are mean±Standard error (SE) of triplicate measurements. Means with unshared superscript on the same row are significantly different ($p<0.05$). A: Soxhlet extraction method, B: Conventional extraction method, C: Cold solvent extraction method, D: Cold press extraction method, R: Resistant.

Based on the responses of the tested pathogens to *C. schweinfurthii* oils an average oil concentration of 6.25% was sufficient as the minimum inhibitory concentration to achieve antibacterial effectiveness, particularly for solvent-extracted and conventionally extracted oils as observed in this study (Table 3). For antifungal efficacy, oil concentrations above 12.5% were required to effectively inhibit filamentous, spore-forming, and budding fungal species. This aligns with recent reports that plant oils rich in unsaturated and medium -chain fatty acids, as well as phenolic compounds, are effective natural antimicrobials (Afolayan *et al*., 2023; Bai *et*

al., 2021). Results revealed that all the extracted oils were not able to kill any of the tested microbial isolates and this was materialized by no inhibition value for the MBC. From the result, the soxhlet extracted oil was ranked as the oil with the most antimicrobial efficacy, followed by its solvent extracted counterpart. Cold press extracted oil recorded the least antimicrobial efficacy closely following the oil extracted via the conventional method. These findings support the potential of *C. schweinfurthii* oil as a natural, plant-based bio-preservative for effective reduction of post-harvest losses, extension of shelf life, and a safer alternative to synthetic chemical preservatives (Afolayan *et al*., 2023; Olagunju *et al*., 2022).

Table 3: MIC and MBC of extracted *C. schweinfurthii* oils

	A		B		C		D	
Test microbes	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	0.39	-	1.56	-	1.56	-	0.78	-
Klebsiella pneumoniae	1.56	-	6.25	-	>1.56	-	>12.5	-
Pseudomonas aeruginosa	>3.13	-	R	-	12.5	-	R	-
Staphylococcus aureus	1.56	-	>3.13	-	3.13	-	6.25	-
Aspergillus flavus	3.13	-	12.5	-	>6.25	-	>12.5	-
Penicillium sp.	≥6.25	-	>12.5	-	12.5	-	>12.5	-
Saccharomyces sp.	1.56	-	3.13	-	3.13	-	6.25	-

A: Soxhlet extraction method, B: Conventional extraction method, C: Cold solvent extraction method, D:Cold press extraction method, MIC: Minimum Inhibitory Concentration, MBC: Minimum Biocidal Concentration, R: Resistant, (-): Not Biocidal.

CONCLUSIONS

The study concludes that the soxhlet method is the most efficient for extracting oil from *C. schweinfurthii* fruits, yielding the highest quantity of oil and exhibiting significantly remarkable antimicrobial effectiveness. While the extracted oil shares comparable refractive index, specific gravity, and density values with some edible oils, their high peroxide, Iodine, FFA, and acid values indicate high susceptibility to oxidative rancidity and a limited shelf life. Furthermore, the significant antimicrobial properties demonstrated by the extracted oils, most especially the soxhlet extracted oil, highlight their potential as natural bio-preservatives, offering a viable alternative to synthetic preservatives for the post-harvest management of agricultural commodities.

AUTHOR CONTRIBUTION

OAF, BEI, LIO conceptualized ideas and BEI supervised the research study. OAF, FTA extracted the oils. LIO, AIG collected and analyzed data. All authors wrote the manuscript. LIO, AIG and IAO revised the manuscript.

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