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Research Article

In vitro and *In vivo* Antioxidant Effect of Mango, Coconut and Cotton Seed Oils on Hydrogen Peroxide- Induced Oxidative Stress in Wistar Rats

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ABSTRACT

This study is aimed at determining the antioxidant activity of Mango kernel oil (MKO), Coconut oil (CCO) and Cotton seed oil (CSO) in vitro and in vivo. Antioxidant parameters demonstrated by five spectrophotometric methods such as: DPPH, FRAP, MCA, HRSA and SRSA was determined in the oils. Superoxide dismutase activity (SOD), catalase activity (CAT) and malondialdehyde (MDA) were evaluated in the serum of the Wistar rats. The antioxidant assay of the oils showed Coconut oil (75.67±0.21 %) was able to neutralize the DPPH radical more than the other oils followed by Cotton seed oil (38.70±0.23 %) and Mango kernel oil (31.56±0.24 %). Coconut oil exhibited the highest FRAP activity at (1.04 mMolFe2+) while the Cotton seed oil displayed significantly lower (P< 0.05) FRAP activity at (0.18±0.00 mMolFe2+). The Metal chelating activity (MCA) of Coconut oil (52.72±0.24 %) was found to be significantly higher than Mango and Cotton seed oils, Superoxide radical scavenging activity showed Coconut oil (62.36±0.01 %) having a relatively high ability to scavenge superoxide radicals better than the other oils with the standard glutathione being significantly higher (81.36±0.07 %). The result of the in vivo study showed the mean values for the SOD of MKO (118.10±5.39 IU/L), CCO (120.53±4.53 IU/L) and CSO (108.80±3.33 IU/L), CAT of MKO (108.93±11.60 IU/L), CCO (96.85±11.69 IU/L) and CSO (88.28±10.66 IU/L) and MDA of MKO (0.25±0.07 mmol/L), CCO (0.24±0.14 mmol/L) and CSO (0.31±0.02 mmol/L). The oils markedly reduced the amount of MDA while significantly increasing the activities of both CAT and SOD content.

Keywords: Antioxidant, Oils, Mango Kernel, Cottonseed, Coconut, *In vivo*, *In vitro*

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INTRODUCTION

Free radicals are extremely unstable and reactive compounds, atoms, or ions that have one unpaired electron in their outermost shell. Reactive oxygen species include singlet oxygen (O_2), superoxide (O_2-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH) are examples of free radicals (Andonova *et al*., 2015). Additionally, there are sulfur species like sulfur dioxide and thiyl radicals, reactive nitrogen species like nitric oxide and peroxynitrite and other free radicals like lipid peroxyl radicals, alkoxy radicals, carbon-centered radicals, iron and copper-free radicals that can be linked to increased oxidative stress and free radical formation, which can disrupt the redox balance (Andonova *et al*., 2015). Free radicals are a natural byproduct of metabolism (Sato *et al*., 2013; Navarro-Yepes *et al*., 2014), They are also created when our cells use food and oxygen to create energy, as well as when we are exposed to microbes, toxins, heavy exercise, alcohol, smoke, ionizing and

UV radiation, pesticides and ozone. In addition to the free radicals produced during physiological processes, heavy metal and other toxicant poisoning can also cause the unregulated creation of free radicals (Jabs, 1999).

An imbalance between the generation of free radicals and the cell's ability to eliminate them is known as oxidative stress. For example, an excess of hydroxyl radical and peroxynitrite can lead to lipid peroxidation, which damages cell membranes and lipoproteins. This can then result in the formation of conjugated diene compounds and malondialdehyde, which are known to be both mutagenic and cytotoxic. Oxidative stress refers to the detrimental consequences of reactive oxygen species that result in biological damage. When ROS production increases, oxidative stress is generated. Oxidative cellular stress is experienced by cells when the balance between oxidative stress producers such as antioxidants and free radicals is upset. According to Preiser (2012), free radicals create oxidative damage that leads to the production of superoxide radicals, peroxynitrite, and numerous other radicals. These radicals are the primary cause of age-associated disorders such as Alzheimer's, Parkinson's, neurodegenerative diseases and other related disorders, as well as how we age.

Reactive oxygen species can be stabilized by antioxidant substances. These are the kinds of compounds that readily oxidize and scavenge free radicals (Hasan and Jat, 2017). Any chemical that substantially inhibits or delays the oxidation of an oxidizable substrate when present in lower quantities is referred to as a biological antioxidant (Sies, 2020). A perfect antioxidant should be easily absorbed by the body, chelate redox metals at physiologically appropriate concentrations, or stops or inhibit the production of free radicals. It ought to influence gene expression favorably and function in membrane or aqueous environments (Sies, 2020).

Free radical scavengers, often known as antioxidants, are substances that interact with and neutralise free radicals to stop them from damaging biological systems' cells. Some of the antioxidants needed by the body to counteract free radicals are produced by it. Endogenous antioxidants are the name given to these antioxidants. But in order to get the remaining antioxidants it requires, the body also depends on external (exogenous) sources, principally from diet. Dietary antioxidant is the term used to describe these exogenous antioxidants. Rich dietary antioxidant sources include fruits, vegetables and grains (Onoja *et al.*, 2014). An intricate system of endogenous antioxidant defence, comprising low molecular weight scavengers like uric acid, coenzyme Q and lipoic acid, as well as endogenous antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione proteins, meticulously maintains cellular redox homeostasis. The intricate human antioxidant defense system must lower reactive oxygen species levels while preserving their beneficial functions in cell signaling and redox control (Halliwell, 2011).

Manufacturers have turned their focus from synthetic to natural antioxidants due to toxicological concerns, stringent laws prohibiting the use of synthetic antioxidants, the carcinogenic potential of some synthetic antioxidants and consumer preferences. The majority of these natural antioxidants are derived from fruits, vegetables, spices, cereals, and herbs which are the richest sources of antioxidant components such as vitamins A, C, E, beta carotene and essential minerals (Yashin *et al*., 2017). Furthermore, the demand for sustainable supplies and ecologically friendly production is pressuring the food and pharmaceutical industries to follow suit (Berger 2009). Since the safety of synthetic antioxidants has been questioned due to their toxic effects during long-term intake, it is reasonable that they should be replaced with natural alternatives that are considered safer and with wild range of health benefits.

A rising number of clinical trials using one or more synthetic antioxidants have shown no benefit from antioxidant supplementation, in several cases, the synthetic antioxidants had the opposite impact (Poljsak *et al*., 2013). Several studies conducted in the last 20 years have demonstrated that oxidative stress can be mitigated by extracting several natural antioxidants from inexpensive resources (Patil, 2013; Lourenco *et al*., 2019). Several edible plants and natural antioxidants have been used in preventing oxidative stress and reducing the incidence of many diseases. The main advantage of using natural compounds or extracts is their milder action compared with those of chemically synthesized drugs (Rahim *et al.*, 2013). Numerous studies have demonstrated the connection between oxidative stress and the development of a number of illnesses, including diabetes, cancer, metabolic disorders, atherosclerosis and cardiovascular diseases (Liguori *et al*., 2018). According to Pepe *et al*. (2017), oxidative stress and its concomitant factors have grown in importance as a public health problem. This has

necessitated the need to carry out researches on natural antioxidants that are safe and milder in action which will help to mitigate the harmful effects of oxidative stress.

A number of studies have investigated the possible utilization of discarded mango seeds in the production of mango seed kernel oil. Mango seed kernel oil is also known as mango kernel fat or, mango butter. Kernels from the stone of mango (*Mangifera indica L*) are usually separated from outer leather by decortications and dried to evaluate the oil content. Mango kernel oil (MKO) may be defined as oil fraction extracted from stone of mango fruit (Kaphueakngam *et al.*, 2009). Although mango oil can sometimes be semi-soft at room temperature, mango oil has a nice, fluid consistency. It is antioxidant, vitamin and mineral rich (Kittiphoom, 2012b)

Included in the Arecaceae family, the coconut tree (Cocos nucifera) is a tall palm that grows to a height of 30 meters and under ideal circumstances, can produce up to 75 fruits annually (Pradeepkumar, 2008). Coconut oil is a vegetable oil extracted from Coconut palm (*Cocos nucifera L*.). Coconut is the most extensively grown and used palm in the world with approximately 12 million hectare in cultivation (FAO, 2014) serving as a major source of income and food for about 10 million families from over 80 countries (Perera *et al*., 2010).

Cotton is one of the most significant commercial crops in the world, it once served as the primary natural supply of fiber and as the foundation of the textile industry, it has a huge impact on the industrial economy. However, the kernel of Cotton seeds contains a sizable amount of oil. Cottonseed oil concentration ranges from 12 to 25 % (Mert *et al*., 2004). Cottonseed oil is produced as a by-product, it is typically used in cooking oil, salad oil and vegetable oil combinations (Metin *et al*., 2003).

Studies from other authors have shown that exposure to H_2O_2 is an effective technique for inducing oxidative stress in animals. H_2O_2 can cause elevation of OH• via the Fenton reaction: $Fe2^{+}+H_2O_2 \rightarrow Fe3^{+}+^{\bullet}OH + OH^{-}$. Hence, this research determined the antioxidant activity of Mango, Coconut and Cotton seed oils *in vitro* and their ability to ameliorate oxidative stress *in vivo*.

MATERIALS AND METHOD

Reagents

All the reagents used in the study were of analytical grade and produced by British Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc. U.S.A.

Experimental Animals

Thirty albino rats were obtained and housed at the animal house of college of Health Sciences, Benue State University Makurdi to acclimatize for two weeks before commencement of experiment. The animals were fed with standard commercial pellet growers feed (Vital Feed, Nigeria) and allowed free access to clean drinking water *ad libitum*.

Sample collection

Local variety of ripe Mango seeds with pH of 5.9 (Chuwpev in Tiv) were collected from Gboko Local Government Area in Benue State, the Cotton seed and Coconut were purchased from Sabon Gari Market in Kano State and Wadata Market in Makurdi, Benue State respectively. They were identified and authenticated at the Herbarium of the Department of Biological Sciences, Benue State University, Makurdi, Nigeria.

Sample Preparation

The Mango seeds underwent a three days sun drying process after which the kernels were extracted by hand. After being diced, the kernels were dried for 12 h at 50 °C, leaving 7 % w/w of moisture. To get a very high yield, the thin cover was separated from the kernel using a tray to blow away the cover. To avoid oxidation, the dry material was ground into a powder using a stainless steel grinder, sealed in a plastic container, and kept in the freezer until extraction (Sikdar *et al*., 2017).

The coconuts were cleaned and dishevelled, the size was reduced by hand shredding them into fine, uniform-sized particles (Okene and Evbuomwan, 2014). The coconut meat was ground and kept until extraction after being oven-dried at 60 °C to a moisture level of 7 % (Amri, 2011).

After being acid-treated, the cotton seeds were sun-dried for three days. Using a nutcracker, the cottonseed shells were slightly fractured at the tip before being manually removed and the kernels separated. The dirt and any other foreign objects were physically removed from the kernels before grinding. Using a stainless steel grinder, the samples were crushed into a powder. To reduce caking and

separation by particle size, each sample was mixed in between grindings (Quampah *et al*., 2012).

Extraction of oil from mango seed kernel

The kernel powder (50 g) was placed in the thimble and about 300 mL of *n*-hexane was poured into the 500 mL round bottom flask. The apparatus was heated at 70°C and allowed to stay for 8 h under continuous extraction using Soxhlet apparatus. At the end of the extraction, the resulting mixture *(miscella*) containing the oil was distilled off to recover solvent from the oil. The total yield obtained was expressed in percentage (AOAC, 2000; Mas' ud *et al.*, 2017).

$$\% \text{ Oil yield} = \frac{\text{weight of oil}}{\text{Weight of sample}} \text{ x } 100$$

Extraction of oil from coconut kernel

The kernel powder (100 g) was placed in the thimble and about 300 mL of *n*-hexane was poured into the 500 mL round bottom flask. Thermal cycle was done at 70°C for 8 h using Soxhlet apparatus. At the end of the extraction solvent was recovered using a rotary evaporator at 40°C under vacuum. The total yield obtained was expressed in percentage (AOAC, 2000; Ixtaina *et al.*, 2011).

$$\% \text{ Oil yield} = \frac{\text{weight of oil}}{\text{Weight of sample}} \text{ x } 100$$

Extraction of oil from cotton seed

The kernel powder (100 g) was placed in the thimble and about 300 mL of *n*-hexane was poured into the 500 mL round bottom flask. The apparatus was heated at 70°C and allowed to stay for 8 h under continuous extraction using Soxhlet apparatus. At the end of the extraction, the resulting mixture *(miscella*) containing the oil was distilled off to recover solvent from the oil. The total yield obtained was expressed in percentage (AOAC, 2000, Mas' ud *et al.*, 2017).

$$\% \text{ Oil yield} = \frac{\text{weight of oil}}{\text{Weight of sample}} \text{ x } 100$$

Evaluation of Antioxidant Activity

1, 1-diphenyl-1-picrylhydrazyl hydrate free radical scavenging activity (DPPH)

The free radical scavenging ability of the samples was determined using the stable radical DPPH (1,1-diphenyl-1-picrylhydrazyl hydrate) method described by (Pownall *et al.*, 2010). The samples/standard glutathione were made to 10 mg /1 mL final concentration using 0.1 M phosphate buffer at pH 7.0 and hydrated for 1 h and the mixture was centrifuged to obtain the clear supernatant. One millilitre (1 mL) of the sample solution was mixed with 1 mL of 0.3 mM DPPH in 95 % methanol. The mixture was vortexed for 60 seconds and incubated in the dark for 30 min. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm against a DPPH control which contained only 1 mL of DPPH solution and 1 mL of 95 % methanol. The buffer was used as blank. The free radical scavenging ability was calculated using the equation below.

$$\% \quad \text{DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \quad x \quad 100$$

Metal Chelating Assay

The metal-chelating assay was carried out according to the method of (Xie *et al.*, 2008). Solutions of 2mM $FeCl_2 \cdot 4H_2O$ and 5mM ferrozine was diluted 20 times (1 mL of each of the solutions made up to 20 mL with distilled water separately). Samples and standard (glutathione) were dissolved to 100 µg/ mL in distilled water. One millilitre of the samples or glutathione (GSH) was mixed with 1mL $FeCl_2 \cdot 4H_2O$ solution. After 5 min incubation, the reaction was initiated by the addition of ferrozine solution (1mL). The mixture was shaken vigorously and after a further 10 min incubation period, the absorbance of the solution was measured spectrophotometrically at 562 nm. Water was used in place of sample. The inhibition of ferrozine–Fe^{+2} complex formations was calculated using the formula:

$$\text{Chelating effect} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where

$A_{control}$ = Absorbance of control sample (the control contained 1 mL each of $FeCl_2$ and ferrozine, complex formation molecules)

A_{sample} = Absorbance of sample.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP of the samples were determined using colorimetric method of (Benzie & Strain, 1999). A 300mmol/L acetate buffer of pH 3.6, 10mmol/L 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mmol/L $FeCl_3.6H_2O$ was mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. Samples or the standard was dissolved in water to a final concentration of 10 mg/mL. A 50 µL aliquot of each of the sample concentrations or the standard was mixed separately with 1mL of FRAP reagent. The mixture was vortex and absorbance was measured at

593 nm, 10 min after incubation. Iron II sulphate calibration was made into different concentrations between 0.25-0.025 mg/mL and then plotted against the absorbance to obtain the calibration curve. The FRAP of the samples were obtained from the calibration curve of iron II sulphate solutions and expressed in mMol Fe^{2+}.

Hydroxyl Radical Scavenging Activities

The Hydroxyl radical scavenging activity (HRSA) of samples was determined using the method described by (Ajibola *et al.*, 2011) with modifications. About 1 g of samples and 3 mM of 1,10-phenanthroline were separately dissolved in 0.1 M of phosphate buffer (pH 7.4) while 3 mM of FeSO4 and 0.01 % (w/v) hydrogen peroxide were each separately dissolved in distilled water. The mixture was kept at room temperature for 1 h and then centrifuged for 30 min. Fifty microliters (50 µL) of the samples or GSH standard were first added to a test-tube followed by 50 µl each of the 1, 10-phenanthroline and FeSO4. To initiate the Fenton reaction in the wells, 50 µL of hydrogen peroxide was added to the mixture and incubated at 37°C for 1 h with constant shaking. The blank consisted of 50 µL phosphate buffer instead of the sample. Absorbance of the colored reaction mixtures were measured at a wavelength of 536 nm.

$$\text{HRSA} = \frac{\text{absorbance of blank for HRSA} - \text{absorbance of sample for HRSA}}{\text{absorbance of blank for HRSA}} \text{ x } 100$$

Superoxide Radical Scavenging Activities

The method described by (Xie *et al.*, 2008) was used to determine Superoxide radical scavenging activity (SRSA) of samples. Sample was extracted using 0.1 M of phosphate buffer to concentration of 10 mg/mL and 1 mL was dissolved in 50 mM of Tris–HCl buffer, pH 8.3 containing 1 mM of EDTA followed by the transfer of 80 µl into a test-tube while 80 µl of buffer was added to the blank. This was followed by addition of 40 µL of 1.5 Mm pyrogallol (dissolved in 10 mMHCl) into each test-tube in the dark and the change in the rate of reaction was measured immediately at room temperature spectrophotometer at a wavelength of 420 nm. The SRSA was calculated using the following equation:

$$\text{SRSA} = \frac{\text{absorbance of blank for SRSA} - \text{absorbance of sample for SRSA}}{\text{absorbance of blank of SRSA}} \text{ x } 100$$

Experimental Method

Thirty healthy Albino Wister Rats (140-180 g) were used for this experiment. The animals were assigned randomly into 6 groups. The six groups consisted of 5 Rats each. The weight of the animals was checked during distribution to ensure a weight difference of +/- 5 g inter and intra cages. After acclimatization, the experimental rats were divided into 6 groups of 5 rats each: Group 1 served as negative control (NC), Group 2–6 were inoculated by oral administration of 0.1 mL/kg body weight of 5 % v/v of H_2O_2. Group 2 that served as positive (H_2O_2) control was not treated. Group 3-6 received 0.1 mL of H_2O_2 and 0.2 mL of Mango Kernel oil, Coconut oil, Cotton seed oil and Vitamin E from days 1 to 14 respectively. Groups 6 served as standard (Vitamin E). The rats were sacrificed under mild chloroform anaesthesia on day 15. An overnight fasting blood sample was collected from each rat through the jugular veins in plain tubes. The samples collected in plain tubes were allowed to clot for 15 min and thereafter centrifuged at 2500 rpm for 10 min. (Okagu *et al.*, 2020). Serum from each sample was subjected to lipid peroxidation and antioxidant status. Approval was obtained from the ethical committee on the use of experimental animals of the College of Health Sciences, Benue State University Makurdi before commencement of experiment.The ethical approval of the present work is CREC/THS/005.

In Vivo Antioxidant Assay

Superoxide Dismutase Activity

SOD was assayed according to the method of (Sun *et al.,* 1988). In this method, xanthine-xanthine oxidase system was used to generate a superoxide flux, and nitroblue tetrazolium (NBT) was used as an indicator of superoxide production. SOD activity was then measured by the degree of inhibition of the reaction unit of enzyme providing 50 % inhibition of NBT reduction. Results are expressed as U/mL.

Estimation of Catalase Activity

The catalase activity in serum was determined with slight modification method Serum (10 μL) was added to test tube containing 2.80 mL of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 mL of fresh 30 mM hydrogen peroxide and the decomposition rate of hydrogen peroxide was measured at 240 nm for 5 min on a spectrophotometer. A molar extinction coefficient of 0.041 mM^{-1} cm^{-1} was used to calculate catalase activity (Atawodi *et al.*, 2003).

Determination of the Lipid Peroxidation (LPO) in Serum

The level of thiobarbituric acid reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method as described by (Draper and Hadley 1990). The serum (50 μL) was deproteinized by adding 1 mL of 14 % trichloroacetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min to complete the reaction and then cooled on ice for 5 min. After centrifugation at 2000 g for 10 min, the absorbance of the colored product (TBARS) was measured at 535 nm with a UV spectrophotometer. The concentration of TBARS was calculated using the molar extinction coefficient of malondialdehyde (1.56 × 105 mol/L/cm) using the formula

A = ΣCL, where A absorbance
Σ = molar coefficient
C = concentration
L = path length
The results were expressed in nmol/mg of protein.

Data Analysis

Data was presented as mean value ± standard deviation of triplicates and analysed by multiple factor analysis of variance (ANOVA) and correlation analyses using SPSS version 21. Multiple comparisons (*post hoc* Duncan multiple range test) was used to evaluate significant differences of the data at $P \leq 0.05$ confidence limit.

RESULT

***Invitro* Antioxidant Activity**

Antioxidant activity of Mango kernel, Coconut kernel, Cotton seed and Vitamin E oils such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating activities (MCA), superoxide radical scavenging activity (SRSA), hydroxyl radical scavenging assay (HRSA), and ferric reducing antioxidant power (FRAP) are presented in Table 1.

***Invivo* Antioxidant Assay**

The results of the *in vivo* antioxidant assay, which includes measurements of malondialdehyde, superoxide dismutase activity and catalase activity on the serum from albino rats are shown in Table 2.

Table 1: *In vitro* Antioxidant Properties of Mango, Coconut and Cotton seed oils

Samples	DPPH (%)	FRAP (mMolFe2+)	Metal Chelating Activities (%)	Hydroxyl Radical Scavenging Activities (%)	Superoxide Radical Scavenging Activities (%)
Mango kernel oil	31.56 ±0.240[a]	0.21 ±0.003[a]	39.50 ±.064[a]	18.46 ±.075[a]	25.78 ±.144[a]
Coconut oil	75.67 ±0.209[b]	1.04 ±0.004[a]	52.72 ±0.237[b]	48.44 ±0.090[b]	62.36 ±0.055[b]
Cotton seed oil	38.70 ±0.225[c]	0.18 ±0.003[b]	44.58 ±0.231[c]	25.77 ±0.100[c]	34.74 ±0.167[c]
Glutathione	95.32 ±0.430[d]	3.13 ±0.085[c]	85.68 ±0.219[d]	78.51 ±0.277[d]	81.36 ±0.072[d]

Values are reported in triplicate determinations
Values are of mean ± standard deviation. Different superscripts between columns depict significant difference ($p \leq 0.05$)

Table 2: *In vivo* Antioxidant assay of Wistar Rats treated with Mango, Coconut, Cotton seed and Vitamin E oils

Group	Malondialdehyde (mmol/L)	Catalase (IU/L)	Superoxide dismutase (IU/L)
Negative Control	0.32±1.60[a]	103.05±4.08[b]	118.45±10.4[b]
Positive Control	0.37±0.04[b]	86.55±10.48[a]	100.28±5.43[a]
Mango kernel oil	0.25±0.07[a]	108.93±11.60[b]	118.10±5.39[b]
Coconut kernel oil	0.24±0.14[a]	96.85±11.69[b]	120.53±4.53[b]
Cotton seed oil	0.31±0.02[a]	88.28±10.66[a]	108.80±3.33[a]
Vitamin E	0.28±0.07[a]	103.88±9.53[b]	123.38±3.98[b]

Values represent means of triplicate values ± sð (standard deviation)
Mean in the same column with different superscript are significantly different at ($p<0.05$)

DISCUSSION

The *in vitro* antioxidant activities of the oils determined by the spectrophotometric method are presented in Table 1. Due to the complexity of phytochemicals and oil derived from seeds, at least two systems must analyse the oil's antioxidant activities to verify its authenticity (Tenore *et al*., 2011). For this reason, five spectrophotometric techniques DPPH, FRAP, MCA, HRSA, and SRSA were used to demonstrate the antioxidant activity of Mango, Coconut, and Cotton seed oils.

A compound's capacity to pair with a radical's unpaired electron determines its capacity to scavenge DPPH radicals (Park *et al*., 2008).

When the commercial standard antioxidant glutathione (95.32) was compared to the antioxidant assay of the oils, the results showed that for DPPH, Coconut oil (75.67 %) was able to neutralize the DPPH radical more than the other oils followed by Cotton seed oil (38.70 %) and Mango kernel oil (31.56 % For ferric reducing antioxidant power (FRAP) Coconut oil (1.04 mMol$Fe2^{+}$) had a better reducing ability (reducing $Fe3^{+}$ to $Fe2^{+}$). While for metal chelating activities (MCA) Coconut oil (52.72 %) had a better ability to bind to metals compared to the other oils and for hydroxyl radical scavenging activity (HRSA) Coconut oil was able to inhibit hydroxyl radical activity by (48.44 %) and this value is higher than those observed in the other oils. Superoxide radical scavenging activities showed Coconut oil (62.36 %) having a relatively high ability to scavenge superoxide radicals better than the other oils. DPPH, MCA, HRSA, and SRSA assay of the oils are significantly different at ($p\leq 0.05$) except for FRAP where there was no significant difference between Mango kernel oil and Coconut oil at ($p\leq 0.05$).

Other researches evidenced that Mango kernel oil contains phenolic compounds including chlorogenic acid, caffeic acid, quercetin and mangiferin which are responsible for its antioxidant activity (Nadeem *et al*., 2016). The antioxidant activity of Coconut oil was attributed to its phenolic content (Marina *et al*., 2009).

Lipid-soluble antioxidants such as tocopherols are naturally occurring in most vegetable oils and are capable of scavenging free radicals. Isomers of tocopherols (*in vitro and in vivo*) have different antioxidant activity. *In vitro* antioxidant activity of tocopherols prevents unsaturated fatty acids from oxidation whereas, in vivo, tocopherols also known as Vitamin E homologues inhibit inflammatory damage, proliferation, and cellular tissues from oxidation (Baümler *et al*., 2017).

It has been discovered that cottonseed oil contains a high concentration of tocopherols, which adds to its antioxidant qualities (Ghazani *et al*., 2016). Cotton seed oil was shown to lower oxidative stressors and inflammatory cytokines (Park *et al*., 2019). Pumpkin seed oil was also reported to exhibit excellent antioxidant activity due to its rich tocopherols, unsaturated fatty acids, and phytosterol content. The antioxidant activity of Tomato oil was reported in the literature (Ameh *et al*., 2023).

The in vitro antioxidant assay of the oils revealed its effective antioxidant activity however Coconut oil performed better and in close comparison with the commercial antioxidant glutathione, which was employed as the reference standard.

Anti-oxidants play defense mechanisms and promote activities for the metabolism of xenobiotics in living systems. They are free radical scavengers that interact and degrade free radicals, thus preventing them from causing cellular damage (Maritim *et al.*, 2003). The In vivo antioxidant activity showed the oils increased the concentration of superoxide dismutase, and catalase and decreased lipid peroxidation.

Result for in vivo antioxidant assay (Table 2), showed that the oils significantly elevated the activity of both catalase and superoxide dismutase content with marked decrease in the malondialdehyde level. There was a significant ($P<0.05$) increase in the catalase activity of Mango kernel oil treatment group when compared to the control group and also a significant increase in the superoxide dismutase activity of the Coconut oil treatment group. Catalase an antioxidant enzyme, catalyzes the breakdown of hydrogen peroxide, a reactive oxygen species generated from normal aerobic metabolism and also known to be a toxic compound (Kohen & Nyska, 2002). Increase in free radicals and imbalance in the normal redox state of tissues can lead to reduction of catalase activity (Awney, 2011).

Superoxide dismutase catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen thereby reducing the likelihood of superoxide anion reacting with nitric oxide to form reactive peroxynitrite.

There is a growing interest in the antioxidant properties of many herbs and spices that were reported to be effective in retarding the process of lipid peroxidation in oils and fatty acids (Yashin *et al*., 2017). Malondialdehyde is known as a secondary

product of lipid peroxidation and is used as a marker of tissue damage resulting from many chain reactions (Gülcin, 2012; Gulcin and Beydemir, 2013). Increase in malondialdehyde concentration has been linked with increased lipid peroxidation which in turn signals development of oxidative stress, cellular and DNA damage. Malondialdehyde concentration was significantly ($P<0.05$) lower in the group treated with the oils compared to the control. Decrease in Malondialdehyde was lower in Coconut oil treated group followed by Mango kernel oil treated group.

The increased serum activities of catalase and superoxide dismutase as observed in this study suggest that the oil had an in vivo antioxidant activity and is capable of ameliorating the effect of reactive oxygen species in biological system. Some of the phytochemical constituents of the oils may be responsible for the antioxidant activities as demonstrated in this study.

CONCLUSION

Most researchers are now looking for natural antioxidants with milder actions which do not have any negative side effects on animal and human health. The *in vitro* antioxidant activities of Mango, Coconut and Cotton seed oils were tested by five assays, which revealed their ability to inhibit the oxidation of useful components by inactivating free radicals, chelating prooxidative metals and reducing reactive species. The oils significantly elevated the activity of both catalase and superoxide dismutase content with marked decrease in malondialdehyde level *in vivo*. In this research the oil with the most effective scavenging ability among the three oils under study was Coconut oil. These oils could be useful for edible purposes and for some industrial applications in foods and pharmaceutical preparations to replace the synthetic antioxidants which are considered to be toxic.

Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES

Agarwal, D. K., Singh, P., Chakrabarty, M., Shaikh, A., and Gayal, S. (2003). Cottonseed oil quality, utilization and processing.

Ajibola, C. F., Fashakin, J. B., Fagbemi, T. N., and Aluko, R. E. (2011). Effect of peptide size on antioxidant properties of african yam bean seed (sphenostylis stenocarpa) protein hydrolysate fractions. *International journal of molecular sciences,* 12(10): 6685-6702

Amri, I.N (2011). The Lauric (Coconut and Palm Kernel) Oil. Vegetable Oils in Food Technology. Wiley-Blackwell. 169-197.

Andonova, L., Georgieva, M., and Zlatkov, A. (2015). Free radicals, oxidative stres, and diseases associated with them. *Pharmacia,* 62(2):26.

Atawodi, S., Bulus, T., Ibrahim, S., Ameh, D., Nok, A., Mamman, M., and Galadima, M. (2003). In vitro trypanocidal effect of methanolic extract of some nigerian savannah plants. *African Journal of Biotechnology,* 2(9):317-321.

Awney, H. A. (2011). Oxidative stress biomarkers in young male rats fed with stevioside. *African Journal of Biochemistry Research,* 5(11):333-340.

Benzie, I. F. F. and Szeto, Y. T. (1999) Total Antioxidant Capacity of Teas by the Ferric Reducing/ Antioxidant Power Assay. *Journal of Agriculture and Food Chemistry*. 47(2):633-636.

Benzie, I. F., and Strain, J. (1999). [2] ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In Methods in enzymology, *Elsevier,* 299:15-27

Berger, R. G. (2009). Biotechnology of flavours—the next generation. *Biotechnology letters,* 31(11):1651.

Bubonja-Sonje M., Giacometti J. and Abram, M. (2011) Antioxidant and antilisterial activity of olive oil, cocoa and rosemary extract polyphenols. *Journal of Food Chemistry*, 127:1821-1827.

Draper, H., and Hadley, M. (1990). Oxygen radicals in biological systems part b: Oxygen radical. *Antioxidant,* 186:421-431.

FAO (2014). FAOSTAT, FAO, ROME. Retrieved April 17, 2024, from http://www.fao.org/faostat/en/#data/QC

Gülcin, I. (2012). Antioxidant activity of food constituents: An overview. *Archives of toxicology, 86*, 345-391.

Gulcin, I., and Beydemir, S. (2013). Phenolic compounds as antioxidants: Carbonic anhydrase isoenzymes inhibitors. *Mini reviews in medicinal chemistry,* 13(3), 408-430.

Halliwell, B. (1999). Detection of free radicals and other reactive species: Trapping and fingerprinting; assays of total antioxidant activity. *Free radicals in biology and medicine*, 422-424.

Halliwell, B. (2011). Free radicals and antioxidants–quo vadis? *Trends in pharmacological sciences,* 32(3):125-130.

Jabs, T. (1999). Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochemical pharmacology,* 57(3):231-245

Joel, J., Sheena, O., Martins, O., Onyemauche, N., and Emmanuel, A (2017). Comparative antioxidant capacity of aqueous and ethanol fruit extracts of tetrapleura tetraptera. *Journal of Biological Sciences*, 17(4):185-193

Kaphueakngam, P., Flood, A., and Sonwai, S. (2009). Production of cocoa butter equivalent from mango seed almond fat and palm oil mid-fraction. *Asian Journal of Food and Agro-Industry, 2*(4):441-447.

Kelsey, N., Hulick, W., Winter, A., Ross, E., and Linseman, D. (2011). Neuroprotective effects of anthocyanins on apoptosis induced by mitochondrial oxidative stress. *Nutritional Neuroscience,* 14(6):249-259.

Mert, M., Akışcan, Y., and Gencer, O. (2004). Inheritance of Oil and Protein Content in some Cotton Generations. *Asian Journal of Plant Sciences*. 3(2):174-176).

Muchiri, D. R., Mahungu, S. M., and Gituanja, S. N. (2012). Studies on mango (mangifera indica, l.) kernel fat of some kenyan varieties in meru. *Journal of the American Oil Chemists' Society,* 89(9):1567-1575.

Navarro-Yepes, J., Zavala-Flores, L., Anandhan, A., Wang, F., Skotak, M., Chandra, N., Li, M., Pappa, A., Martinez-Fong, D., and Del Razo, L. M. (2014). Antioxidant gene therapy against neuronal cell death. *Pharmacology & Therapeutics,* 142(2):206-230.

Okene, E., & Evbuomwan, B. (2014). Solvent extraction and characterization of oil from coconut seed using alternative solvents. *Int J Eng Tech Res,* 2:135-138.

Patil, D. (2013). Role of antioxidants in stability of edible oil. *Trends Post-Harvest Technology,* 1:68-73

Perera L, Perera S.A.C.N., Bandaranayake C.K., and Harries H.C. (2010). Coconut. In: J. Vollmann and I. Rajcan (Eds.). Oil Crops. Springer, Dordrecht, Heidelberg, London, New York. 369-396

Pownall, T. L., Udenigwe, C. C., and Aluko, R. E. (2010). Amino acid composition and antioxidant properties of pea seed (pisum sativum l.) enzymatic protein hydrolysate fractions. *Journal of Agricultural and Food Chemistry,* 58(8):4712-4718.

Pradeepkumar, T. (2008). Management of horticultural crops. 11: New India Publishing.

Preiser, J. C. (2012). Oxidative stress. *Journal of Parenteral and Enteral Nutrition,* 36(2): 147-154.

Quampah, A., Huang, Z. R., Wu, J. G., Liu, H. Y., Li, J. R., Zhu, S. J., and Shi, C. H. (2012). Estimation of oil content and fatty acid composition in cottonseed kernel powder using near infrared reflectance spectroscopy. *Journal of the American Oil Chemists' Society,* 89(4):567-575.

Rahim, S. M., Taha, E. M., Mubark, Z. M., Aziz, S. S., Simon, K., and Mazlan, A. (2013). Protective effect of cymbopogon citratus on hydrogen peroxide-induced oxidative stress in the reproductive system of male rats. *Systems biology in reproductive medicine,* 59(6):329-336.

Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical interventions in aging,* 2(2): 219.

Sato, H., Shibata, M., Shimizu, T., Shibata, S., Toriumi, H., Ebine, T., Kuroi, T., Iwashita, T., Funakubo, M., and Kayama, Y. (2013). Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. *Neuroscience,* 248:345-358.

Sies, H., Stahl, W., and Sundquist, A. R. (1992). Antioxidant functions of vitamins: Vitamins e and c, beta-carotene, and other carotenoids a. *Annals of the New York Academy of Sciences,* 669(1):7-20.

Sikdar, D. C., Siri, H., Vinay, S., Varsha, S., and Rao, R. (2017). Solvent extraction of mango (mangifera indica l.) seed kernel oil and its characterization. *International Journal of Technical Research and Applications,* 5(4):43-47.

Sun, Y., Oberley, L. W., and Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical chemistry,* 34(3):497-500.

Tenore, G. C., Ciampaglia, R., Arnold, N. A., Piozzi, F., Napolitano, F., Rigano, D. and Senatore, F. (2011) Antimicrobial and antioxidant properties of the essential oil of Salvia lanigera from Cyprus. *Food Chemistry and Toxicology*, 49:238-43

Xie, Z., Huang, J., Xu, X., and Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry,* 111(2):370-376.

Yashin, A., Yashin, Y., Xia, X., and Nemzer, B. (2017). Antioxidant Activity of Spices and Their Impact on Human Health: A Review. Antioxidants, 6(3):70.