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Comparative Study of Free Radical Scavenging and Lipid Peroxidation Inhibition Potentials of *Oxythenanthera Abyssinica* (Rhizomes) Sourced from South Eastern Nigeria

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ABSTRACT

Oxytenanthera abyssinica rhizomes have been used in the treatment of aliments varying from colics, diabetes, polyuria and albuminiura, these ethanomedicinal potencies have been attributed to its rich phytochemical contents and free radical scavenging capacity. This work is aimed at investigating the effect of crude oil pollution on total phenolic, flavonoids and free radical scavenging potentials of methanol extract of *Oxytenanthera abyssinica* rhizomes. The ability of the plant extracts to scavenge 2,2-diphenyl- 1- picylhydrazyl (DPPH), hydroxyl radical ($\cdot OH$), superoxide radical (O_2^{-}), nitric oxide radical ($NO^{\cdot}$) and ability to inhibit lipid peroxidation was assayed following standard methods, spectrophotometric method was used to assay for total phenolic and flavonoid content. Non crude oil polluted *O. abyssinica* extract (NCOPOAE) and crude oil polluted *O. abyssinica* extract(COPOAE) in a concentration dependent manner showed a potent antioxidant activity in DPPH radical scavenging assay (EC_{50}= 1.03 ± 0.01μg/ml) and (EC_{50} = 1.04 ± 1.01μg/ml), inhibited hydroxyl radical (EC_{50} = 1.17 ± 1.02μg/ml) and (EC_{50} = 1.46 ± 1.21μg/ml), superoxide anion radical (EC_{50} =1.30 ± 1.21μg/ml) and (EC_{50} =1.51 ± 1.42μg/ml) and nitric oxide (EC_{50} = 1.40 ± 1.23μg/ml) and (EC_{50}= 1.53 ± 1.42μg/ml) also inhibited non enzymatic lipid peroxidation (EC_{50} = 1.58 ± 1.42μg/ml) and (EC_{50} = 2.47 ± 2.13μg/ml) and had more of phenolic and flavonoid contents than COPOAE. This result indicates that NCOPOAE contains more antioxidant compounds than COPOAE, which could make it more potent in ethanomedicinal use.

Keywords: *Oxytenanthera abyssinica rhizomes, crude oil pollution, antioxidant, lipid peroxidation, reactive oxygen species*

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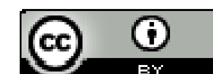

1. INTRODUCTION:

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during regular metabolism and energy generation in the body (Mavi *et al.*, 2004). They have been shown to play important role in performing physiological roles such as signaling molecules, regulation of signal transduction and gene expression, activation of receptor and nuclear transduction (Valko *et al.*, 2006). However, the imbalance between reactive oxygen species and nitrogen species and the antioxidant system results to oxidative stress. They cause oxidative damage to DNA, proteins and lipids and eventually leading to many chronic diseases, such as aging, diabetes, cancer and other degenerative diseases in humans (Maxwell, 1995.; Harman, 1998). *Oxytenanthera abyssinca* (bamboo) is a tropical drought resistant plant that grows in open lowlands, grassland and highlands, though typically on hills or along intermittent watercourses (Sharma and Sarma, 2013). In ethnomedicine, the rhizome is used in the treatment of dysentery and the leaves are marketed for treating diabetes, colics and rheumatism. In Senegal leaf decoctions are taken to treat oedema, polyuria and albuminuria (Louppe *et al.*, 2008).

Crude oil being one major pollutant has been described as a compound mixture of over 6000 different hydrocarbons and metals (Edward, 1989). It has been shown to be harmful to health and results to damage of biodiversity, pollution of land and damage to ecosystem (Suleiman, 1987). Disruption of biochemical processes, decrease in water and nutritional uptake are among the frequent responses exhibited by plants that are affected by crude oil pollution (Eriyamremu and Asagba, 2007). We have therefore investigated the free radical scavenging capacity, lipid peroxidation inhition, phenolic and flavonoid content of methanol extract of (NCOPOAE) and (COPOAE) in other to elucidate the effect of crude oil on radical scavenging potentials, phenolic and flavonoid content of the extract.

2. MATERIALS AND METHODS

2.1. Chemicals

L-ascorbic acid, and 2,2-diphenyl-1- picrylhydrazyl (DPPH.) radical were purchased from Fluka Chemicals, 2 deoxy-Dribose, sodium nitroprusside (SNP), sodium nitrite, sulpanilamide, phosphate buffer saline (PBS), phosphoric acid, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), ferric chloride ($FeCl_3^+$), naphthylethylenediamine dihydrochloride, ethylenediaminetetraacetic acid (EDTA), nitro blue tetrazolium (NBT), sodium carbonate (Na_2CO_3), aluminium trichloride, perchloric acid ($HClO_4$), butylated hydroxyltoluene (BHT), polyvinylpolypyrrolidone, riboflavin, ferrous sulphate, hydrogen peroxide (H_2O_2), thiobarbituric acid (TBA), Folin - ciocalteu reagent (FCR) and trichloroacetic acid (TCA) were all purchased from Sigma Chemical Co.(St. Louis, MO).

2.2. Preparation of plant crude extract

The rhizomes of *O. abyssinica* were collected from Owerezukala of Orumba Local Government Area of Anambra State. The Oil-polluted *O. abyssinica* rhizome samples were collected from Akirika Ndoki in Ukwa East Local Government Area of Abia State Nigeria. The rhizomes were authenticated in Nnamdi Azikiwe University Awka by the taxonomist, air dried at room temperature and by milling it was reduced to powder. The powder was extracted with 80% methanol and concentrated with rotary eveaporator and stored at 4^0C until being used.

2.3. Estimation of total phenolic content of NCOPOAE and COPOAE

Total phenolic content in *O. abyssinica* rhizome extract was determined according to the method described by Singleton and Rossi Jr (1965). Estimation of phenolic content of the extracts was done by folin-ciocalteu method, where phenolic compounds form a blue complex. Gallic acid was used as standard solution. 0.5 mL of test extract solution was mixed with 2.5 mL of 1N folin-ciocalteu reagent and incubated for 5 minutes and then 2 mL of 75 g/L sodium carbonate was added followed by distilled water. After incubation at room temperature for 2 hours, absorbance of reaction mixture was measured at 760 nm against blank as methanol. The total phenolic content was expressed in *μ*g of gallic acid equivalent (GAE) of dry plant material.

2.4. Estimation of total flavonoid content of NCOPOAE and COPOAE

Total flavonoid content in *O. abyssinica* rhizome extract was determined colorimetrically according to the method described by Lamaison and Carret (1990). By using quercetin as standard, 5 mL of 2% aluminum chloride in methanol was mixed with the same volume of test solution, after incubation for 10 minutes; absorbance was measured at 415 nm against blank sample. A standard curve of quercetin at 0–50 *μ*g/mL was prepared and used to determine the total flavonoids. The average of three readings was used and then expressed in *μ*g quercetin equivalent flavones per mg extract.

2.5. Antioxidant activity assays

In vitro DPPH radical - scavenging assay

The method reported by Gyamfi *et al.* (1999) as described by Awah *et al.*, (2010) was used to assay for the capacity of the extracts to scavenge DPPH radical. Briefly, a 2.0 ml solution of the extract at different concentrations (15.65 – 250μg/ml) diluted two-fold in methanol was mixed with 1.0 ml of 0.3 mM DPPH. in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with a UV-visible spectrophotometer. DPPH. Radical inhibition was calculated using the equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample. The EC_{50} represented the concentration of the extract that inhibited 50% of radical.

In vitro hydroxyl radical (·OH) Scavenging Assay

The method of Aruoma and. Halliwell, (1987). Was used to assay for hydroxyl radical, different concentrations (15.65 – 250μg/ml) of the extracts were added to a reaction mixture containing, the following final concentrations of reagents in a final volume of 1.0 ml: 2-deoxyribose (2.5 μM), potassium phosphate buffer (pH 7.4, 20 mM), $FeCl_3$ (100 μM), EDTA (104 μM), H_2O_2 (1 mM), and L-ascorbic acid (100 μM). The mixtures were incubated for 1 h at 37 ^{o}C, followed by addition of 1.0 ml of 1% (w/v) TBA in 0.05 M NaOH and 1.0 ml of 2.8% (w/v) TCA. The resulting mixture was heated for 15 min at 100°C. After cooling on ice, absorbance was measured at 532 nm. Inhibition of 2-deoxyribose degradation expressed in percentage was calculated as per the equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

A_0 = Absorbance of the control, A_s = Absorbance of the test sample.

In vitro superoxide radical ($O_2^{\cdot-}$) Scavenging Assay

The capacity of the extracts to inhibit the formazan formation upon photochemical reduction of nitroblue tetrazolium (NBT) was assayed according to the method of Martinez *et al*, (2001). Each 3.0 ml reaction mixture contained 0.05 M PBS (pH 7.8), 13 mM methionine, 2 μM riboflavin, 100 μM EDTA, NBT (75 μM) and 1.0 ml of test sample solutions of different concentrations of (15.83 - 250μg/ml). The tubes were kept in front of a fluorescent light (725 lumens, 34 watts) and absorbance was read at 560 nm after 20 min. The inhibition of superoxide anion was calculated using the equation.

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

A_0 = Absorbance of the control, A_s = Absorbance of the test sample.

In vitro nitric Oxide (NO·) scavenging Assay.

The ability of the extracts to scavenger nitric oxide was done according to the method described by Sreejayan and Rao, (1997). In a final volume of 3mL, the reaction mixture contained: 10 mM SNP in 0.5M phosphate buffer (pH 7.4)

and various concentrations (15.63–250 μg/mL) of the NCOPOAE and COPOAE. After incubation for 60min at 37^0C, Griess reagent (0.1% α-napthyl-ethylenediamine in water and 1% sulphanilic acid in 5% H_3PO_4) was added. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with α-napthylethylenediamine were measured spectro photo metrically at 540 nm. Ascorbic acid was used as a positive control. Nitric oxide scavenging ability (%) was calculated by using the equation.

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

A_0 = Absorbance of the control, A_s = Absorbance of the test sample.

2.6. Lipid Peroxidation Assay

A modified thiobarbituric acid – reactive species (TBARS) assay Banerjee *et al.*, (2005) was used to measure the lipid peroxide formed using egg yolk as lipid- rich media Ruberto *et al.*, (2000). Egg homogenate was prepared with phosphate buffered saline, PH 7.4 at a ratio of 10% v/v after which 500µl of the solution and 100µl of the sample in different dilution of 2- fold (15.63 - 250µg/ml) were added to a test tube and made up to 1.0ml with distilled water, then 50µl of Fe_2SO_4 (0.075M) and 20µl of L- ascorbic acid (0.1M) were added and incubated at 37^0C for 1hr to induce lipid peroxidation. 0,2ml of EDTA (0.1M) and 1.5ml of TBA reagent (3g TBA, 120g TCA and 10.4ml 70% $HClO_4$ in 800ml of distilled water) were added there after in each sample and heated for 15 minutes at 100^0C, after cooling, samples were centrifuged for 10min and absorbance of the samples supernatant was measured at 532nm and % inhibition was calculated with the following equation:

$$\% \text{ inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

A_0 = Absorbance of the control, A_s = Absorbance of the test sample.

2.7. Statistical analysis

The results were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 for Windows. All the data are expressed as mean ± SEM (n = 3). Student's T-test was used to compare means, and values were considered significant at $p < 0.05$.

3. RESULT

3.1. Quantitative Estimation of Totatl phenol and flavonoid content of NCOPOAE and COPOAE

Total phenolic content was expressed in μg of gallic acid equivalent. Poly phenolic and hydroxyl compounds may contribute directly to antioxidative action. The total phenolic content was 2360.2 ± 13.10mg/100 g for NCOPOAE and 2348.1± 96.2 mg/100g for COPOAE of gallic acid equivalent per mg extract. All values are expressed in mean ± SEM (n = 3), (Table 1). While the total flavonoid content of NCOPOAE and COPOAE were 781.8 ± 28.10 mg/l and 339.1± 16.9 mg/dl of quercetin equivalent per mg plant extract. All values are expressed in mean ± SEM (n = 3).

3.2. Inhibitory effect of NCOPOAE and COPOAE on DPPH

The addition of DPPH extracts at various concentrations of the extracts resulted in rapid discoloration, showing good scavenging capacity of the extracts. The extracts scavenged DPPH in a dose dependent manner with highest percentage inhibition at 90.64 and 89.88 (figure 1) with EC_{50} of 1.03 ± 0.01 µg/ml for NCOPOAE and 1.04 ± 1.01 µg/ml for COPOAE.

3.3. Inhibitory effect of NCOPOAE and COPOAE on hydroxyl radical

The extracts inhibited hydroxyl radical – induced deoxyribose degradation in a concentration dependent manner (figure 2). The effective concentrations (EC_{50}) values of the extract were 1.17 ± 1.02 µg/ml and 1.46 ± 1.12 µg/ml respectively.

3.4. Inhibitory effect of NCOPOAE and COPOAE on superoxide radical

Addition of NCOPOAE and COPOAE to formazan solution at 560 nm resulted in a decrease in the absorbance of the blue formazan solution, as a result of the ability of the extract to scavenge $O_2^{\cdot -}$ radical generated from the photochemical reduction of riboflavin. As shown in figure 3, the activity was concentration dependent with EC_{50} of 1.30 ± 1.21 µg/ml for NCOPOAE and 1.51 ± 1.42 µg/m for COPOAE.

3.5. Innibitory effect of NCOPOAE and COPOAE on nitric oxide radical

In this study NCOPOAE and COPOAE in SNP caused a strong concentration dependent inhibition of nitric oxide radical (figure 4), with NCOPOAE preventing the formation of nitrite more than COPOAE, EC_{50} 1.40 ± 1.23 µg/ml for NCOPOAE and 1.53 ± 1.42 µg/ml for COPOAE. The extracts had efficient $NO^{\cdot}$ Scavenging activity at 250 µg/ml concentrations.

3.6. Inhibitory effect of NCOPOAE and COPOAE on lipid peroxidation

The inhibition of Fe^{2+} - induced lipid peroxidation was assayed by TBARS formation assay, NCOPOAE showed a concentration dependent inhibition of lipid peroxidation with EC_{50} 1.58 ± 1.42 µg/ml than COPOAE EC50 2.47 ± 2.13 µg/ml (Figure 5).

Table1: Total phenolic and flavonoid content of NCOPOAE and COPOAE

Extracts	Totalphenol (mg/100g)	Total flavonoid (mg/dl)
NCOPOAE	2360.2 ± 13.10	781.8 ± 28.10
COPOAE	2348.1 ± 96.20	339.1 ± 16.90

Data represented as mean ± SEM (n = 3)

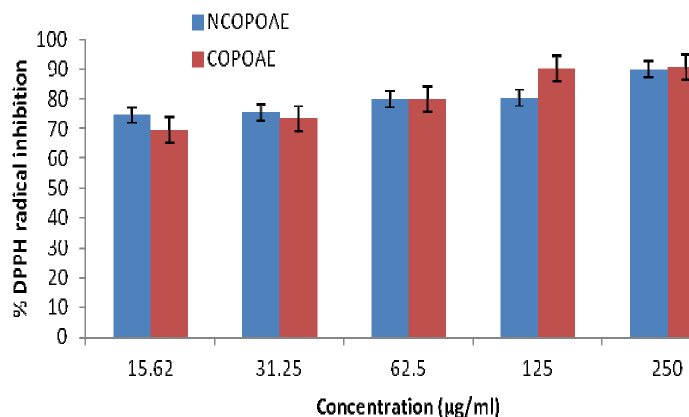

Figure1: Inhibition of DPPH radical by non crude oil polluted *O.abyssinica* (NCOPOAE) compared to crude oil polluted *O.abyssinica* (COPOAE). The results are expressed as mean ± SEM (n=3).

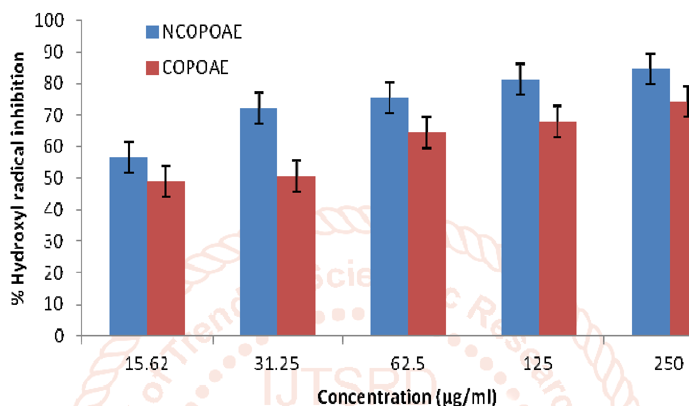

Figure2: Inhibitory effect of non crude oil polluted *O.abyssinica* (NCOPOAE) on hydroxyl radical compared to crude oil polluted *O.abyssinica* (COPOAE). Results are expressed as mean ± SEM (n=3).

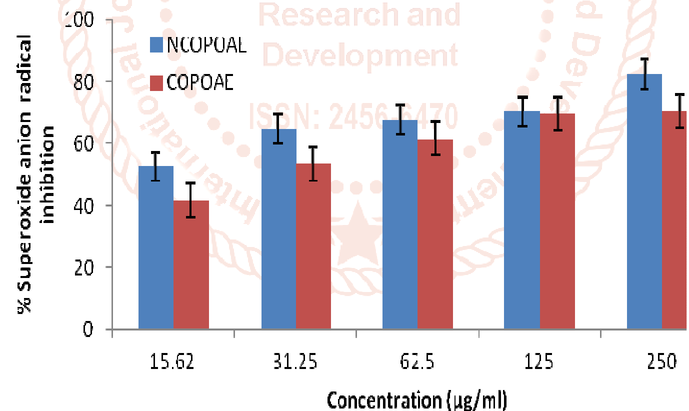

Figure3: Scavenging effect of non crude oil polluted *O.abyssinica* (NCOPOAE) on superoxide anion radical compared to crude oil polluted *O.abyssinica* (COPOAE). The results are expressed as mean ± SEM (n=3).

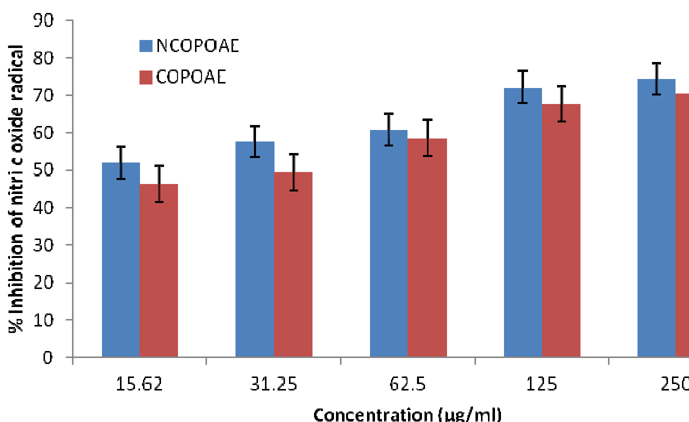

Figure4: Scavenging effect of non crude oil polluted *O.abyssinica* (NCOPOAE) on nitric oxide radical compared to crude oil polluted *O.abyssinica* (COPOAE). Results are expressed as mean ± SEM (n=3).

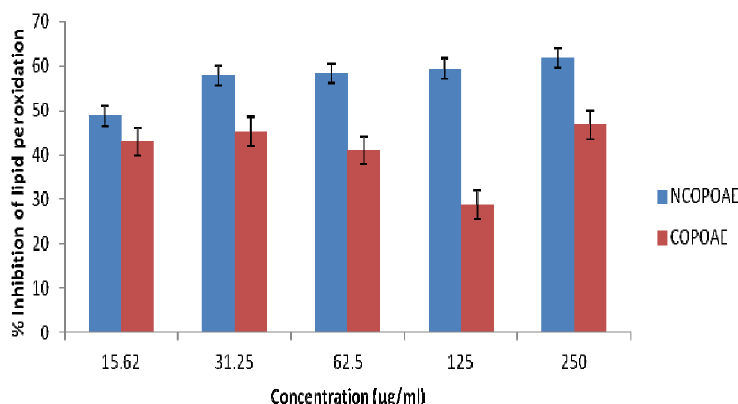

Figure 5: Inhibitory effect of non crude oil polluted *O.abyssinica* (NCOPOAE) on lipid peroxidation induced by Fe^{2+} /ascorbate system compared to crude oil polluted *O.abyssinica* (COPOAE). Results are expressed as mean ± SEM (n=3).

Discussion

Antioxidants are compounds capable of either delaying or inhibiting the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species (Pisoschi and Negulescu, 2011). Due to the complex nature of phytochemicals, a single method to evaluate the antioxidants activity cannot be evaluated. In this context, different standard methods were used to ascertain the capacity of NCOPOAE and COPOAE to scavenge free radicals.

Phenolics and flavonoids have been shown to act as primary antioxidants, the quench free radicals either by donating hydrogen atom or electron to oxidants, from this study, COPOAE had a lesser phenolic and flavonoid content compared to NCOPOAE (Table 1), which correlates with the observations of Iwuala and Odjegba (2017) that crude oil pollution affected the quantity of secondary metabolites in plants. The lower concentration of phytochemicals observed in COPOAE could be related to limited carbon assimilation caused by crude oil, that may consequently affect carbon allocation for secondary metabolites synthesis as the synthesis of the basic skeleton for the active secondary metabolite is dependent on carbon assimilation during photosynthesis (Zobayed *et al*., 2007). Net photosynthesis decreases when plants are under stress such as high temperature, high salinity, heavy metal and oil pollution (Murch *et al*., 2003).

The addition of the extracts to diphenylpicrylhydrazine (DPPH) resulted to the discoloration of DPPH from purple to yellow, which could be as a result of the antioxidant compounds NCOPOAE and COPOAE neutralizing the radical nature of DPPH either by transferring electron or single hydrogen atom to DPPH. The antioxidant compounds in NCOPOAE scavenged the radical more than COPOAE (figure 1).

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and biological damage like reduction of disulphide bonds in proteins, resulting in their unfolding and scrambled refolding into abnormal spatial configurations (Lipinski, 2011). The antioxidant compounds in NCOPOAE, competed with deoxyribose against ⁻OH radical generated from Fe^{3+} dependent system and prevented the reaction more than COPOAE (figure 2). The antioxidants in the extracts could be acting as Fe^{3+} chelator in the system or donating hydrogen atom and accelerating the conversion of H_2O_2 to H_2O (Wang *et al*., 2007).

Superoxide anions (O_2^-) radical is also harmful to the cellular components (Muller *et al*., 2007). NCOPOAE and COPOAE were observed to be efficient scavengers of superoxide anion radical by inhibiting the formation of reduced nitroblue tetrazolium (NBT) in a concentration dependent manner (figure 3). NCOPOAE scavenged the radical more than COPOAE as depicted by the effective concentration (EC_{50}). Polyhydroxyl compounds such as flavonoids and catechins are reported to be effective scavengers of superoxide anion radical (Robak and Gryglewski, 1988). The scavenging potentials of NCOPOAE and COPOAE will possibly depend on the position and number of hydroxyl groups present in the phenolic compounds in the extracts (Khanduja *et al.,* 2006).

Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells and plays an important role in inflammatory process (Mayank *et al*., 2014). Despite its beneficial effects,NO· toxicity increases when it reacts with superoxide to form peroxynitrite anion (·$ONOO^-$), which is a potential strong oxidant that can decompose to form ·OH and NO_2 (Pacher *et al*., 2007). NCOPOAE showed a more potent NO· scavenging activity than COPOAE (Figue 4) The NO· generated from sodium nitropurisde (SNP) reacts with O_2 to form nitrite, the extracts may have inhibited nitrite formation by directly competing with oxygen in the reaction mixture.

The inhibition of lipid peroxidation by NCOPOAE and COPOAE was assessed by the amount of malondialdehyde (MDA) produced using egg yolk as lipid- rich media. NCOPOAE showed a concentration dependent inhibition and more efficacious in inhibiting lipid peroxidation compared to COPOAE (Figure 5). Considering the destructive effect of lipid peroxides in disease conditions, the extracts may afford cytoprotective effect (Muller *et al*., 2007).

Conclusion

The findings of this study shows that crude oil pollution affects the quantity of secondary metabolites such as phenolics and flavonoids in plants, hence affects the free radical scavenging potentials of the affected plant, as these metabolites serves as primary antioxidants.

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