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Antioxidant potential of essential oil from *Phoenix dactylifera* (L.) seed

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Abstract

Oxidative stress has been implicated in the initiation and progression of a plethora of diseases including atherosclerosis, diabetes mellitus, rheumatoid arthritis, neurodegenerative diseases, cardiovascular diseases and cancer. Due to the toxic and mutagenic side effects of some synthetic antioxidant compounds, intense interest is recently converged on natural antioxidants. This study therefore aimed at evaluating the antioxidant potential of essential oil extracted from *Phoenix dactylifera* seed. *Phoenix dactylifera* seed essential oil (PDEO) was extracted via steam-distillation technique. The antioxidant activity of the essential oil was assessed by radical scavenging, lipid peroxidation and reduction power assays. From the results obtained in this study, *P. dactylifera* essential oil showed significant concentration dependent antioxidant potential with maximum activity at 500 µg/ml viz: DPPH radical scavenging (76.24 ± 0.12 %), ABTS radical scavenging (68.97 ± 0.36 %), Nitric oxide radical scavenging (67.86 ± 0.16 %), Hydroxyl radical scavenging (68.70 ± 0.26 %), Fe^{2+} chelating ability (66.74 ± 0.25 %), Ferric reducing antioxidant power (262.29 ± 0.253 mMol FeSO4/g) and inhibition of lipid peroxidation (67.78 ± 0.29 %). Findings from this study revealed that essential oil from *Phoenix dactylifera* seed elicits substantial antioxidant activity and may serve as a veritable pharmacological candidate towards prevention of free radical related pathological damages.

Keywords: *Phoenix dactylifera* Seed; Essential Oil; Free Radicals; Antioxidant; Oxidative Stress

1. Introduction

The burden of Non-communicable diseases (NCDs) such as cancer, cardiovascular diseases, chronic respiratory diseases, neurodegenerative diseases and diabetes is on the increase, accounting for more than two-thirds of annual mortality globally [1]. The occurrence of NCDs has been associated with oxidative stress, where the production of free radicals and the biological antioxidant defense system is not proportional [2]. Free radicals are highly reactive molecules, formed in all tissues during normal cellular metabolism [3]. However, physical stress, deep-fried foods, spicy foods, and diverse environmental factors, including exposures to tobacco smoke, pollutants, ionizing radiations, synthetic pesticides, and solvents are able to increase their production [4]. Free radicals have the propensity of indiscriminately engaging molecules they contact [5]. In pathological conditions, free radical formation is highly elevated resulting in oxidative stress which causes damage to crucial biological molecules like DNA, protein and lipid [6]. In this condition, endogenous antioxidants like glutathione peroxidase, super oxide dismutase and catalase become insufficient for deactivating or balancing of free radicals, as such, external antioxidants through dietary supplementation are necessary to forestall oxidative injury and conserve optimal cellular functions [7, 8]. Antioxidants modulate several oxidative processes innately occurring in tissues. They terminate or impede the oxidation reaction by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors [2].

Natural antioxidants have evoked substantial attention in prophylactic medicine. Plants contain a wide variety of free radical scavenging molecules, such as anthocyanins, vitamins, carotenoids, phenols, dietary glutathione and

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endogenous metabolites. Redox properties of phenolic compounds are responsible for the main antioxidant potentials in herbs and spices, which allow them to act as reducing agents, hydrogen donators and free radical quenchers [9]. In addition, they evoke the cellular synthesis of endogenous antioxidant molecules [10]. Essential oils are complex, hydrophobic mixtures primarily composed of monoterpenes, sesquiterpenes, and their oxygenated derivatives [11]. They are high-value products with a wide variety of impressive biological properties which include antifungal, antibacterial, anticancer, cytotoxic, hematopoietic and allelopathic properties with profound effects on animals, humans, and even other plants [12, 13, 14]. *Phoenix dactylifera*, known as the date palm, is an economically important plant popular in North Africa, South Asia, and the Middle East, belonging to the Arecaceae family [15]. Folkloric medicine noted its parts are used for the treatment of various diseases such as cough, rheumatism, respiratory infections, nephropathy, gastropathy, asthma, cancer and high blood pressure [16].

Due to the toxic and mutagenic side effects of some synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroxyquinone (TBHQ) [17], huge interest is recently focused on natural antioxidants. An *in vivo* study has proven *P. dactylifera* seed essential oil to be non-toxic [14] making it an auspicious candidate for the investigation of likely antioxidant property, since there is a dearth of this information in literature. This study therefore evaluates the antioxidant potential of essential oil from the seed.

2. Methods

2.1. Extraction of Essential oil from *Phoenix dactylifera* Seed (PDEO)

Phoenix dactylifera fruit flesh were manually separated from the endocarp (seed), followed by milling of the seed to obtain a powdery form. Essential oil from the seed was then extracted using the Clevenger steam-distillation method [14].

2.2. Antioxidant Activity

2.2.1. DPPH Radical Scavenging Activity

Free radical scavenging activity of PDEO was evaluated quantitatively using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay as described by Nazir *et al.* [18]. DPPH solution was prepared by dissolving 24 mg of the reagent in 100 mL methanol. A 500 µg/mL stock solution of PDEO was also prepared in methanol and serially diluted to obtain dilution having concentrations 3.91–500 µg/mL. Subsequently 0.1 mL of each dilution was mixed with 3 mL of DPPH solution. The mixtures were incubated for 30 min at 25 °C. Absorbance was measured at wave length 517 nm through UV spectrophotometer (Thermo Electron Corporation; USA) and ascorbic acid was used as a positive control. Percentage DPPH scavenging potential was calculated using the following equation: (%) Free radical scavenging = {(Ac – As)/Ac } × 100

Where: As is the absorbance of DPPH solution after reacting with the essential oil and Ac is the absorbance of DPPH solution with methanol blank.

2.2.2. ABTS Radical Scavenging Activity

The 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radicals scavenging potential of essential oil was determined using the method of Nazir *et al*. [18] with slight modification. ABTS radical cation (ABTS•+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 hours before use. The ABTS•+ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 190 µL of the ABTS radical solution was mixed with 10 µL of varying concentrations (500 to 3.91 µg/mL) of the essential oil dissolved in methanol. After 6 min, the absorbance was measured at 734 nm using UV spectrophotometer. Standard ascorbic acid was used as positive control. All the test samples were performed in triplicates and percentage ABTS scavenging potential was calculated using the formula:

$$(\%) \text{ Free radical scavenging} = \{(Ac - As)/Ac\} \times 100$$

Where: As is the absorbance of ABTS solution after reacting with the essential oil and Ac is the absorbance of ABTS solution with methanol blank.

2.2.3. Hydroxyl Radical Scavenging Activity

This activity was evaluated using the method described by Sethi *et al*. [19]. Sixty microliters of $FeSO_4 \cdot 7H_2O$ (1 mM) was added to 90 µl of aqueous 1,10 phenanthroline (1 mM), 2.4 ml of 0.2 M phosphate buffer (pH 7.8) was added to the above mixture, followed by addition of 150 µl of hydrogen peroxide (0.17 mM) and 1.5 ml of different concentrations of the sample (500 to 3.91 µg/ml). The absorbance of the mixture was read at 560 nm against blank after 5 min. Ascorbic acid was used as the standard. The percentage inhibition was calculated as: % OH radical scavenging capacity = [(Ac – As)/Ac] × 100, where Ac and As are the absorbance values of the blank and the test samples, respectively.

2.2.4. Nitric Oxide (NO) Radical Scavenging Activity

The nitric oxide scavenging activity of oil was determined using a previously reported method Joshi *et al*. [20]. Two millilitres of sodium nitroprusside (10 mM) in phosphate buffer saline pH 7.4 was mixed with different concentrations of sample (3.91–500 µg/mL) and incubated at 25°C for two-and-a-half hours. To the above sample, 1 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2 ml orthophosphoric acid) was added. The absorbance of the chromophore was measured at 546 nm. The standard antioxidant used was ascorbic acid. The nitric oxide radical scavenging percentage was expressed with respect to the control (without the addition of antioxidants) as follows:

% NO scavenging activity = (Ac − As)/Ac × 100. Where: Ac is the absorbance of reaction mixture + vehicle solvent (control); As is the absorbance of the reaction mixture (containing the different concentrations PDEO and Griess reagent).

2.2.5. Ferrous (Fe^{2+}) Ions Chelating Ability

The chelation of Fe^{2+} by the essential oil was evaluated using the method described by Sethi *et al*. [19]. Different concentrations of the essential oil (3.91–500 µg/ml) were first mixed with 1 ml of methanol and 3.7 ml of deionised water. The resulting mixture was allowed to react with 0.1 ml of $FeCl_2$ (2 mM) and 4.2 ml of ferrozine (5 mM) for 10 minutes at room temperature. Then the absorbance was measured at 562 nm. All readings were taken in triplicates and, ascorbic acid was used as standard. The percentage inhibition of ferrozine-Fe^{2+} complex formation (ferrous ion chelating ability) was calculated according to the formula:

(% chelating ability) = [(Ac − As /Ac] × 100, where Ac and As are the absorbance values of the control sample and the test sample, respectively.

2.2.6. Lipid Peroxidation Assay

A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid-rich media with a minor change (methanol used for dilution of the essential oil instead of water) as described by Upadhyay *et al.* [21]. Egg homogenate (250 μL, 10% in distilled water, v/v) and 50 μL of varying concentrations of the essential oil (3.91–500 µg/mL) in methanol were mixed in a test tube and the volume was made up to 500 μL, by adding methanol. Finally, 25 μL $FeSO_4$ (0.07 M) was added to the above mixture and incubated for 30 min, to induce lipid peroxidation. Thereafter, 750 μL of 20% acetic acid (pH 3.5) and 750 μL of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulphate) and 25 μL 20% TCA were added, vortexed, and then heated in a boiling water bath for 60 min. After cooling, 3.0 mL of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured against 3 mL butanol at 532 nm. For the blank 0.1 mL of methanol was used in place of the oil. The percentage of inhibition of lipid peroxide was calculated using the equation: % inhibition = {(Ac – As)}/ (Ac) × 100. Where Ac and As are the absorbance values of the control and the test samples, respectively.

3. Results

3.1. Radical Scavenging Activity

The efficiency of an antioxidant is often connected to its capacity to scavenge stable free radicals. Figures 1, 2, 3, and 4 demonstrate the free radical scavenging activity of PDEO relative to the standard antioxidant (ascorbic acid). As illustrated, PDEO exhibited a dose-dependent scavenging activity, with the following values at 500 µg/ml: DPPH (76.24 ± 0.12%), ABTS (68.97 ± 0.36%), Nitric oxide (67.86 ± 0.16%) and Hydroxyl radical (68.70 ± 0.26 %). The control (ascorbic acid) demonstrated superior radical scavenging activity over PDEO at all concentrations which was most pronounced in the DPPH assay. However, PDEO compared favorably with the control at 250 µg/ml, 62.5 µg/ml, 31.25 µg/ml respectively for ABTS, NO and OH radical scavenging assays.

3.2. Ferric Reducing Antioxidant Power (FRAP)

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric-TPTZ (Fe^{3+}-TPTZ) complex and producing a colored ferrous-TPTZ (Fe^{2+}-TPTZ) complex by a reductant at a low pH. The ability of PDEO to reduce iron (III) to iron (II) was determined and compared to ascorbic acid, as shown in Figure 5. PDEO showed a FRAP value of 238.71 ± 0.349 mMol $FeSO_4$/g at 125µg/ml which was significantly comparable to the value of the control 244.64 ± 0.168 mMol $FeSO_4$/g. However, the FRAP value of the control at 500µg/ml supersedes that of PDEO (262.29 ± 0.253 mMol $FeSO_4$/g) at same concentration.

3.3. Iron Chelating Activity

The capacity to chelate transition metals specifically iron (II) is ascribed a defense mechanism of antioxidant agents. The ability of PDEO to chelate iron (II) (Fe^{2+}) was determined. As shown in figure 6, the results illustrates that PDEO was able to chelate Fe^{2+} in a concentration-dependent manner with maximum Fe^{2+} chelating ability of 66.74 ± 0.25% at 500µg/ml, whereas the control produced 69.23 ± 0.33% chelating ability at the same concentration (p-value <0.05).

3.4. Lipid Peroxidation Activity

The results of the lipid peroxidation inhibition activity of PDEO at various concentrations is shown in figure 7. The lipid peroxide inhibition activity of PDEO (34.72 ± 0.07%) at 15.63µg/ml compared favorably with the standard antioxidant (36.52 ± 0.32%) at same concentration. However, at higher concentrations, the standard antioxidant demonstrated superior lipid peroxide radical scavenging activity than PDEO.

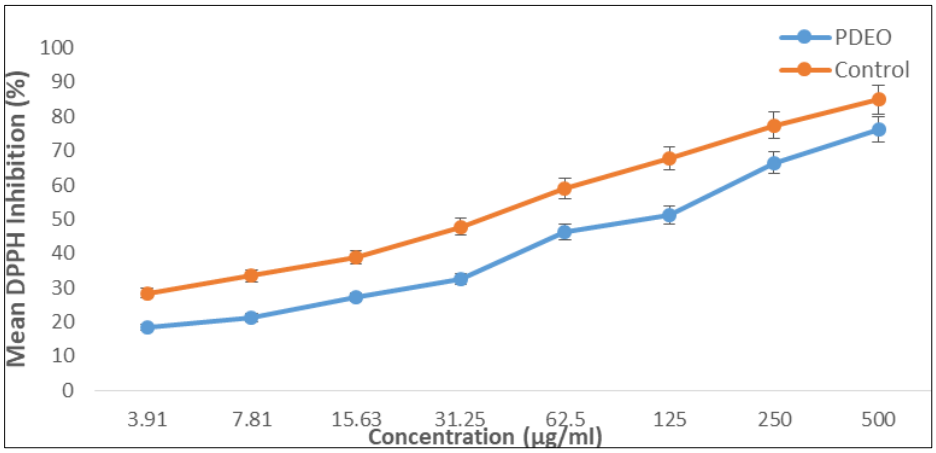

Figure 1 DPPH radical scavenging activity of *Phoenix dactylifera* seed essential oil

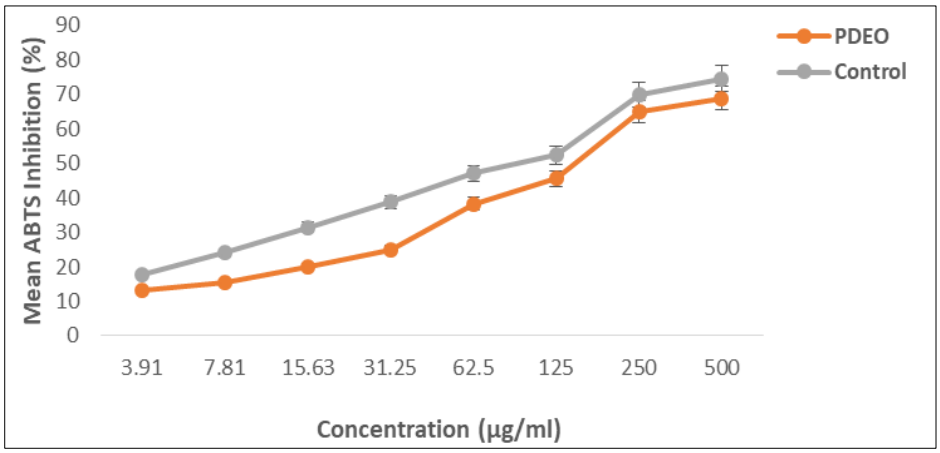

Figure 2 ABTS radical scavenging activity of *Phoenix dactylifera* seed essential oil

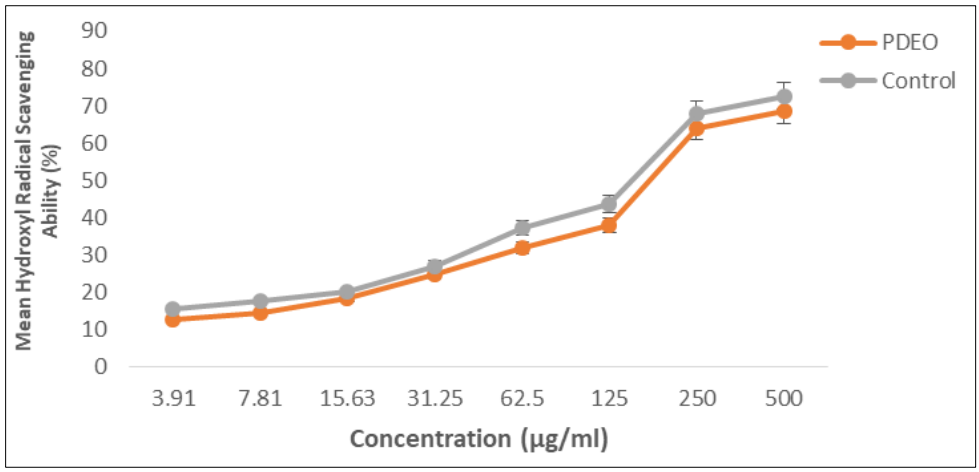

Figure 3 Hydroxyl radical scavenging activity of *Phoenix dactylifera* seed essential oil

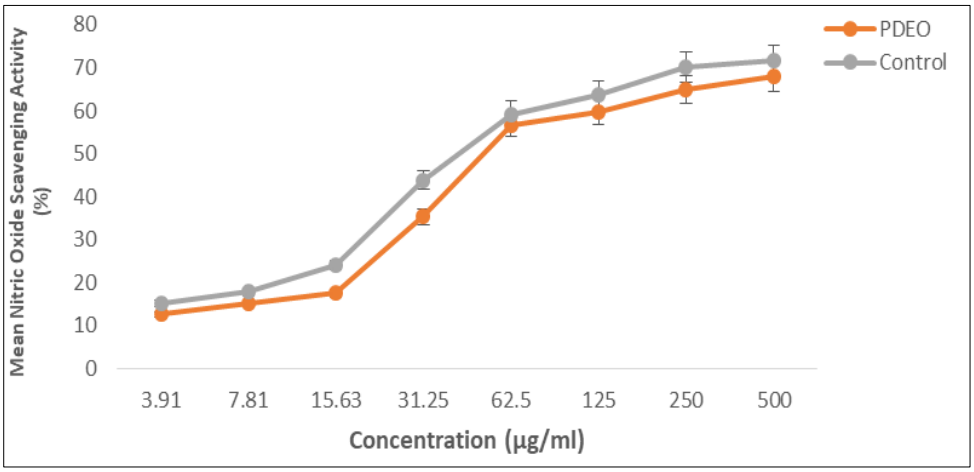

Figure 4 Nitric Oxide radical scavenging activity of *Phoenix dactylifera* seed essential oil

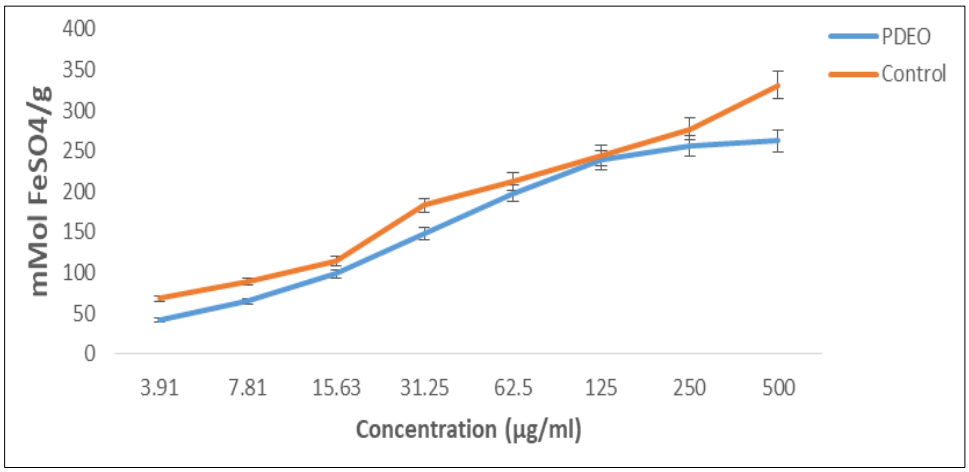

Figure 5 Ferric reducing antioxidant power (FRAP) of *Phoenix dactylifera* seed essential oil

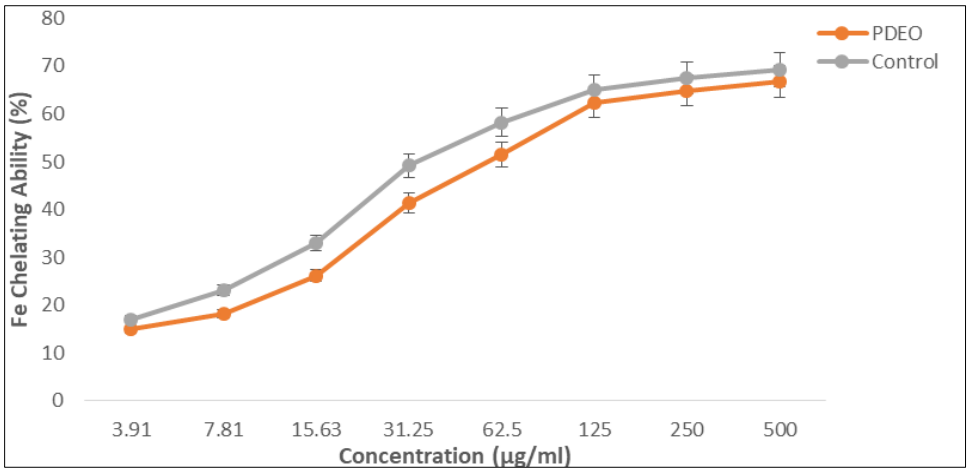

Figure 6 Fe Chelating ability of *Phoenix dactylifera* seed essential oil

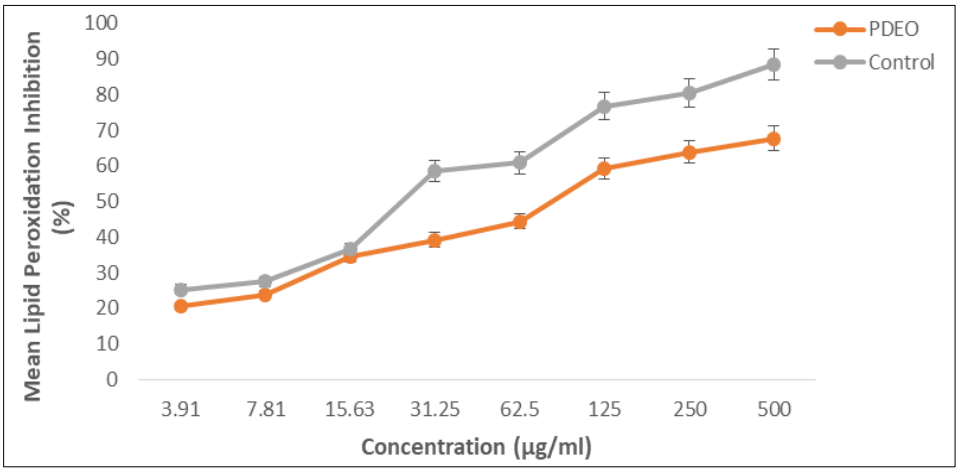

Figure 7 Lipid Peroxidation Inhibition activity of *Phoenix dactylifera* seed essential oil

4. Discussion

Free radicals are paramount initiators of oxidative processes that could lead to a plethora of detrimental cellular effects and pathological conditions. Natural antioxidants possess free radical scavenging ability, as such, they quench the free radical chain of reaction [22, 16]. Essential oil from diverse aromatic plants can protect against oxidative stress by contributing to the host's total antioxidant defense system [23, 24].

Essential oil from *P. dactylifera* seed showed marked antioxidant activity in this study. Several studies have also validated the antioxidant activity (radical scavenging activity) of essential oil from various plants. Nazir *et al*. [18] reported that *Elaeagnus umbellata* essential oil exhibited prominent free radical scavenging activities against DPPH and ABTS radicals with IC_{50} values of 70 and 105 µg/mL respectively. Likewise, Haile *et al.* [25] and Barbosa *et al*. [26] respectively reported notable radical scavenging activities of *Thymus serrulatus* (DPPH- 94.51±0.08%, NO- 34.89 ± 11.88%, OH- 51.35 ± 0.59%) and *Ocimum tenuiflorum* (DPPH- IC_{50} 2.31±0.02 µg/mL, ABTS- IC_{50} 2.22±0.23 µg/mL) essential oils. Factors like stereo-selectivity of the radicals or the solubility of the oil in the different testing systems have been reported to affect the capacity of essential oils in quenching different radicals [27]. Some compounds, which have ABTS radicals scavenging activity, do not exhibit DPPH activity. Hence, the ability of PDEO to scavenge different free radicals in different systems is noteworthy.

Lipid peroxidation, a type of oxidative degeneration of polyunsaturated lipids, has been implicated in a variety of pathogenic processes [28, 29]. Further, lipid oxidation is a crucial concern in the food industry as it can occur throughout processing, storage, and distribution, directly affecting food stability, safety, and quality due to increased oxidative

rancidity, loss of essential fatty acids, generation of off odors and off flavors, and formation of toxic compounds [30,31,32]. PDEO showed significant inhibition of lipid peroxidation in this study. According to Radulescu *et al* [33] *Melissa officinalis* subsp. *officinalis* essential oil exhibited a strong inhibitory effect (94.031 ± 0.082%) in the β-carotene bleaching assay by neutralizing hydroperoxides, responsible for the oxidation of highly unsaturated β-carotene; also, the study of Nartey *et al.* [34] showed good inhibition of lipid peroxidation by *Chrysophyllum albidum* fruit essential oil with IC_{50} value of 457.4 ± 0.3 µg/mL.

The FRAP assay is potentially functional for appraising antioxidant defense system. The FRAP value of PDEO in this study is remarkable. This observation is in consonance with FRAP results of essential oil from various sources. According to Ghosh *et al.* [35], at concentration of 100 µg/mL, essential oil from *Magnolia sirindhorniae* flower showed maximum FRAP value of 70.25 ± 1.33%; while Abba *et al.* [24] reported a FRAP IC_{50} value of 1.02mg/mL for *Englerastrum gracillimum* essential oil.

Iron has been reported to exert pernicious effect on biological macromolecules by reacting with hydrogen peroxide (H_2O_2) and superoxide anion (O_2•) to produce hydroxyl radical (OH•) [36]. These radicals can also lead to the formation of other reactive oxygen species (ROS) [16]. It is conceivable that PDEO acts as a good iron chelator and putatively prevented the oxidation of iron (II), thereby thwarting the generation of hydroxyl radical and impeding oxidative assault in the process. Also, the reducing potential of PDEO could be due to the hydrogen donating capacity of its phyto-constituents which may interact with free radicals to quash radical chain reactions.

5. Conclusion

Accumulation of reactive oxygen species induces oxidative stress which has been implicated in several disease conditions. The phenomenon of oxidative stress can be mitigated by endogenous antioxidants, however, these are sometimes insufficient to counteract the actions of the excess free radicals generated in tissues. The combination of assays employed in this study more accurately reflects the antioxidant potential of PDEO. Findings from this study revealed that essential oil from *Phoenix dactylifera* seed elicits substantial antioxidant activity and may serve as a veritable natural antioxidant source and pharmacological candidate towards prevention of free radical related pathological damages. In *vivo* antioxidant studies are recommended to further affirm the bioactivity of *P. dactylifera* as observed in this study.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

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