The Antioxidant Content and Protective Effect of Argan Oil and Syzygium aromaticum Essential Oil in Hydrogen Peroxide-Induced Biochemical and Histological Changes

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Abstract: Oxidative stress is an important etiology of chronic diseases and many studies have shown that natural products might alleviate oxidative stress-induced pathogenesis. The study aims to evaluate the effect of Argan oil and Syzygium aromaticum essential oil on hydrogen peroxide (H₂O₂)-induced liver, brain and kidney tissue toxicity as well as biochemical changes in wistar rats. The antioxidant content of Argan oil and Syzygium aromaticum essential oil was studied with the use of gas chromatography. The animals received daily by gavage, for 21 days, either distilled water, Syzygium aromaticum essential oil, Argan oil, H₂O₂ alone, H₂O₂ and Syzygium aromaticum essential oil, or H₂O₂ and Argan oil. Blood samples were withdrawn on day 21 for the biochemical blood tests, and the kidney, liver and brain tissue samples were prepared for histopathology examination. The results showed that the content of antioxidant compounds in Syzygium aromaticum essential oil is higher than that found in Argan oil. H₂O₂ increased level of blood urea, liver enzymes, total cholesterol, Low Density Lipoprotein (LDL-C), Triglycerides (TG) and Very Low Density Lipoprotein (VLDL), and decreased the total protein, albumin and High Density Lipoprotein-cholesterol (HDL-C). There was no significant effect on blood electrolyte or serum creatinine. The histopathology examination demonstrated that H₂O₂ induces dilatation in the central vein, inflammation and binucleation in the liver, congestion and hemorrhage in the brain, and congestion in the kidney. The H₂O₂-induced histopathological and biochemical changes have been significantly alleviated by Syzygium aromaticum essential oil or Argan oil. It is concluded that the Argan oil and especially the mixture of Argan oil with Syzygium aromaticum essential oil can reduce the oxidative damage caused by H₂O₂, and this will pave the way to investigate the protective effects of these natural substances in the diseases attributed to the high oxidative stress.

Keywords: hydrogen peroxide; oxidative stress; Argan oil; Syzygium aromaticum essential oil
1. Introduction

Free radicals are naturally present in the living organism, and they include reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. They are produced during metabolism of energy in the cell as a result of the reduction of oxygen with one electron which forms superoxide anion (O$_2$•$^-$) and with 2 or 3 electrons which forms H$_2$O$_2$ by the action of enzymes such as oxidases and with production of hydroxyl radical (OH•). Furthermore, nitric oxide (NO) with anionic superoxide (O$_2$•$^-$) gives peroxynitrite (ONOO$^-$), which is RNS [2]. Pollutions of air and water, toxins, drugs, heavy metals, pesticides, and cigarette smoke play an important role in the production of ROS [3].

When the ROS present in physiological concentration, they play an important role in the maintenance and the functioning of the body, but when their production exceeds the capacity of the cells to trap them, they start a state of oxidation called oxidative stress [4].

When the oxidative stress is moderate, the intervention of endogenous antioxidant systems of the organism can handle the situation to return to the physiological state. However, when oxidative stress becomes chronic, it leads to the appearance of several diseases such as cardiovascular diseases, neurodegenerative diseases, diabetes and cancer [5–11].

Exogenous antioxidants such as vitamin E and C, phenolics, flavonoids, flavonols, flavones and carotenoids have been found to mitigate the activity of the endogenous antioxidant defense and can protect against diseases that result from oxidative stress [12].

Argania spinosa (Sapotaceae) is an endemic tree of south-western Morocco, which gives valuable Argan oil. The extraction of this oil was made by three methods: (i) a traditional method which is very slow and produces oil with an insufficient quality of conservation due to the water added during the process of extraction; (ii) a mechanical press which does not require the addition of water during extraction; and (iii) a solvent extraction method which produces oil with unsatisfactory organoleptic properties compared to the oil extracted by traditional method or by mechanical press. This technique is exclusively used to prepare the oil for cosmetic purposes [13,14].

Argan oil is rich in antioxidant compounds such as caffeic acid, vanillic acid, ferulic acid, resorcinol and catechin [15]. Several studies have shown that Argan oil has beneficial effects against many diseases such as cardiovascular diseases, obesity, cancer, and diabetes [16–20].

Essential oil is an odorous product of organic compounds found naturally in aromatic plants, and it is obtained by hydro distillation, steam distillation, and pressing techniques [21,22]. The clove (Syzygium aromaticum) is a tree from (Myrtaceae) family, and its essential oil has been reported to be one of the strongest essential oil in its antioxidant activity; this is due to the chemical composition especially eugenol.

Several studies have shown that exposure to H$_2$O$_2$ is an effective technique for inducing oxidative stress in animals. H$_2$O$_2$ can cause elevation of OH• via the Fenton reaction: Fe$^{2+}$ + H$_2$O$_2$ $\rightarrow$ Fe$^{3+}$ + •OH + OH$^-$ [23–26].

In this context, the present study was designed to explore the antioxidant content of Argan oil and Syzygium aromaticum essential oil, and to investigate the protective effect of Argan oil administered alone and the effect of the formulation of Syzygium aromaticum essential oil emulsified in Argan oil against the harmful toxicity induced by H$_2$O$_2$.

2. Results

2.1. Chemical Composition of Syzygium aromaticum Essential Oil and Argan Oil

Syzygium aromaticum essential oil was obtained with a percentage of 12.6% (w/w). The chemical composition of Syzygium aromaticum essential oil obtained with Gas chromatography–mass spectrometry (GC/MS) was represented in (Table 1). The result showed that Eugenol (2-Methoxy-4-(2-propenyl) phenol) is the major constituent of the oil with a percentage of 87.03% followed by Eugenyl acetate (4-Allyl-2-methoxyphenyl acetate) with a percentage of 11.25%. The composition of
Argan oil includes Schottenol (159 mg/100 g), Spinasterol (129 mg/100 g), Stigmasta-8,22-dien-3β-ol (12 mg/100 g) and other (27 mg/100 g).

Table 1. Constituents of *Syzygium aromaticum* Essential Oil and their Relative Percentages of Total Chromatogram Area and Kovats Index.

| Compounds          | Kovats Index | Area (%) | Chemical Formula   | Kovats Index (Literature) |
|--------------------|--------------|----------|--------------------|----------------------------|
| Eugenol            | 1353.00      | 87.03    | C_{10}H_{12}O_{2}   | 1327.70 [27]               |
| β-Caryophyllene    | 1428.00      | 0.69     | C_{13}H_{24}        | 1433.90 [28]               |
| Eugenyl acetate    | 1538.00      | 11.25    | C_{12}H_{14}O_{3}   | 1524.00 [29]               |
| Caryophyllene oxide| 1689.00      | <0.10    | C_{13}H_{26}O       | 1606.00 [30]               |

2.2. Antioxidant Content and Activity of *Syzygium aromaticum* Essential Oil and Argan Oil

The results showed that the content of antioxidant compounds in *Syzygium aromaticum* essential oil is higher than that found in Argan oil (Table 2). The Total antioxidant capacity (TAC) of the *Syzygium aromaticum* essential oil is higher than Argan oil.

Table 2. Phenolics, Flavones/Flavonols, Flavonoids and Total antioxidant capacity (TAC) Content.

| Sample                                      | Phenolics 1 | Flavones and Flavonols 2 | Flavonoids 2 | TAC 3       |
|---------------------------------------------|-------------|--------------------------|--------------|-------------|
| Argan oil (mg Eq/100 g)                     | 41.28 ± 0.40 * | 1.80 ± 0.07 *            | 8.31 ± 1.06 * | 90.90 ± 4.53 * |
| *Syzygium aromaticum* essential oil (mg Eq/100 g) | 165.52 ± 9.71 | 29.60 ± 1.02            | 44.08 ± 5.34 | 3235.50 ± 237.40 |

1 equivalent of gallic acid; 2 equivalent of quercetin; 3 equivalent of ascorbic acid. Data are the mean of three replicates (n = 3) and presented as mean ± SD. * Significant as compared to *Syzygium aromaticum* essential oil (p < 0.001).

2.3. Effect of the Interventions on Enzymatic Markers

The result (Figure 1) showed that Argan oil and clove essential oil prepared in Argan oil alleviated the effect of H\textsubscript{2}O\textsubscript{2} on Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT) and aspartate aminotransferase (AST). H\textsubscript{2}O\textsubscript{2} increased level of liver enzymes.

![Figure 1](image-url)  
**Figure 1.** Effect of interventions on Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels. *: comparison between distilled water group and all groups. b: comparison between H\textsubscript{2}O\textsubscript{2} group and all groups. ***/p < 0.05. Data are the means of three replicates (n = 3) and presented as mean ± SD.
2.4. Effect of the Interventions on Total Protein and Albumin Levels

The result showed that H$_2$O$_2$ significantly decreases the total protein and albumin (Figure 2). However, when H$_2$O$_2$ was used with Argan oil or with Argan oil and clove essential oil, there was no significant change in the total protein or albumin as compared to the water group except for total protein in group that received H$_2$O$_2$ with Argan oil.

Figure 2. Effect of the interventions on total protein and albumin levels. a: comparison between distilled water group and all groups. b: comparison between H$_2$O$_2$ group and all groups, c: comparison between H$_2$O$_2$ + Argan oil and H$_2$O$_2$ + Argan oil + clove essential oil. * $p < 0.05$, *** $p < 0.001$. Data are the means of three replicates ($n = 3$) and presented as mean ± SD.

2.5. Effect of the Interventions on TC, TG, LDL-C, HDL-C and VLDL Levels

H$_2$O$_2$ increases the total cholesterol, LDL-C, TG and VLDL and decreases HDL-C, whereas in groups that received Argan oil or Argan oil with essential oil of Syzygium aromaticum, there was no significant change in these parameters (Figure 3).

Figure 3. Effect of interventions on TC, TG, LDL-C, HDL-C and VLDL levels. a: comparison between distilled water group and all groups. b: comparison between H$_2$O$_2$ group and all groups. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Data are the means of three replicates ($n = 3$) and presented as mean ± SD.
2.6. Effect of the Interventions on Serum Electrolytes

H$_2$O$_2$ alone or with use of argan oil or Syzygium aromaticum essential oil with Argan oil did not cause significant changes in the blood electrolytes (Table 3).

Table 3. Effect of Argan Oil and Syzygium aromaticum (clove) Essential Oil on Plasma Electrolytes levels.

| Minerals             | Distilled Water | Argan Oil | Argan Oil + Clove Essential Oil | H$_2$O$_2$ | H$_2$O$_2$ + Argan Oil | H$_2$O$_2$ + Argan Oil + Clove Essential Oil |
|----------------------|----------------|-----------|--------------------------------|------------|------------------------|--------------------------------------------|
| Sodium (mmol/L)      | 140 ± 30       | 139 ± 2.1 | 138 ± 2                        | 143 ± 2    | 142 ± 1.5              | 145 ± 2.1                                  |
| Potassium (mmol/L)   | 6 ± 1          | 5.8 ± 0.8 | 5.6 ± 1.4                     | 6.3 ± 1.2  | 5.6 ± 1.7              | 6 ± 0.9                                    |
| Chloride (mmol/L)    | 103 ± 1.2      | 105 ± 3   | 100 ± 4                       | 106 ± 2.5  | 102 ± 2.3              | 102 ± 2                                    |

Data are the means of three replicates ($n = 3$) and presented as mean ± SD.

2.7. Effect of the Interventions on Blood Urea and Creatinine Levels

H$_2$O$_2$ did not cause a significant change in the plasma creatinine as compared to the control. Blood urea is significantly increased in the group received H$_2$O$_2$, while in groups received H$_2$O$_2$ with Argan oil or Argan oil with Syzygium aromaticum essential oil, there was no change in the blood urea level (Table 4).

Table 4. Effect of the Interventions on Blood Urea and Creatinine Levels.

| Renal Markers     | Distilled Water | Argan Oil | Argan Oil + Clove Essential Oil | H$_2$O$_2$ | H$_2$O$_2$ + Argan Oil | H$_2$O$_2$ + Argan Oil + Clove Essential Oil |
|-------------------|----------------|-----------|--------------------------------|------------|------------------------|--------------------------------------------|
| Creatinine (mg/dL)| 0.7 ± 0.05     | 0.6 ± 0.03| 0.7 ± 0.03                     | 0.75 ± 0.2 | 0.6 ± 0.04             | 0.6 ± 0.08                                  |
| Urea (mg/dL)      | 24 ± 1 ****b   | 22 ± 0.5 ***b | 23 ± 1.5 ***b                    | 30 ± 0.2 **a,***b | 22 ± 1.5 ***b | 21 ± 0.9 ***b                      |

*: comparison between distilled water group and all groups. **: comparison between H$_2$O$_2$ group and all groups. ***p < 0.001. Data are the means of three replicates ($n = 3$) and presented as mean ± SD.

2.8. Effect of the Interventions on Organs Weights

Liver and kidney weights and relative weights were significantly increased in the H$_2$O$_2$ treated group, while brain weight and relative brain weight were significantly decreased (Table 5). The same results were encountered in the group received H$_2$O$_2$ with Argan oil. However, in the group received H$_2$O$_2$ with Argan oil with Syzygium aromaticum essential oil, there was no changes in the kidney weight and relative kidney weight.

Table 5. Effect of the Interventions in the Organs Weights.

| Parameters                     | Distilled Water | Argan Oil | Argan Oil + Clove EO | H$_2$O$_2$ | H$_2$O$_2$ + Argan Oil | H$_2$O$_2$ + Argan Oil + Clove Essential Oil |
|-------------------------------|----------------|-----------|----------------------|------------|------------------------|--------------------------------------------|
| Body weight (g)               | 190 ± 10       | 198 ± 3   | 192 ± 3.3            | 181 ± 2    | 198.5 ± 1.5             | 203 ± 5                                    |
| Brain weight (g)              | 1.87 ± 0.1 ***b| 1.92 ± 0.12 ***b | 1.8 ± 0.15 ***b          | 1.29 ± 0.11 ***b | 1.62 ± 0.04 ***b,***b | 1.71 ± 0.05 ***b,***b                     |
| Liver weight (g)              | 6.45 ± 0.4 ***b| 6.41 ± 0.1 ***b | 6.52 ± 0.1 ***b          | 9.18 ± 0.11 ***b | 6.78 ± 0.05 ***b,***b | 6.65 ± 0.06 ***b,***b                     |
| Kidney weight (g)             | 0.75 ± 0.04 ***b| 0.76 ± 0.02 ***b | 0.735 ± 0.01 ***b        | 1.195 ± 0.01 ***4 | 0.975 ± 0.02 ***4,***a | 0.8 ± 0.02 ***4,***a                      |
| Brain relative weight (g/100 g BW) | 0.984 ± 0.005 ***b | 0.969 ± 0.06 ***b | 0.935 ± 0.077 ***a,***b | 0.71 ± 0.06 ***a | 0.816 ± 0.02 ***a,***b | 0.842 ± 0.02 ***a,***b                     |
| Liver relative weight (g/100 g BW) | 3.394 ± 0.02 ***b | 3.383 ± 0.06 ***b | 3.263 ± 0.05 ***b        | 5.07 ± 0.06 ***a | 3.41 ± 0.025 ***a,***b | 3.27 ± 0.029 ***a,***b                     |
| Kidney relative weight (g/100 g BW) | 0.394 ± 0.002 ***b | 0.383 ± 0.01 ***b | 0.361 ± 0.05 ***b        | 0.660 ± 0.05 ***b | 0.491 ± 0.01 ***b,***b | 0.394 ± 0.009 ***b,***b                     |

*: comparison between distilled water group and all groups. **: comparison between H$_2$O$_2$ group and all groups, ***p < 0.001. Data are the means of three replicates ($n = 3$) and presented as mean ± SD.
2.9. Effects of the Interventions on Histopathological Changes

2.9.1. Brain

Histopathological examination of the brain tissue (Figure 4) showed that H$_2$O$_2$ induces congestion and hemorrhage. However, it did not induce brain hemorrhage when used along with the Argan oil or Syzygium aromaticum essential oil emulsified in Argan oil.

Figure 4. Histopathological evaluation of the brain of the control and stressed groups, the samples were stained with hematoxillin and eosin, the arrows represent pathological changes in tissue: (a) control groups: normal tissue ×200; (b) H$_2$O$_2$ group: congestion ×200; (c) H$_2$O$_2$ group: hemorrhage ×200; (d) H$_2$O$_2$ + Argan group: congestion ×400; (e): H$_2$O$_2$ + Argan + Syzygium aromaticum essential oil group: the yellow arrow represent congestion and the black arrow represent inflammatory cells infiltration ×200.

2.9.2. Liver

Histopathological examination of the liver tissue (Figure 5) demonstrated that H$_2$O$_2$ induces dilatation in the central vein, inflammation and binucleation. However, it induced only dilatation in the central vein when it was co-administered with Argan oil or Syzygium aromaticum essential oil emulsified in Argan oil.

Figure 5. Cont.
emulsified in Argan oil, H$_2$O$_2$ did not cause histopathological change in the kidney tissue. However, when it was co-administered with *Syzygium aromaticum* essential oil emulsified in Argan oil, H$_2$O$_2$ did not cause histopathological change in the kidney tissue.

### 2.9.3. Kidney

Histopathological examination of the kidney tissue (Figure 6) showed that H$_2$O$_2$ induces kidney tissue congestion, however, when it was co-administered with *Syzygium aromaticum* essential oil emulsified in Argan oil, H$_2$O$_2$ did not cause histopathological change in the kidney tissue.

![Figure 5](image5.png)

**Figure 5.** Histopathological evaluation of the liver of the control and stressed groups, samples were stained with hematoxylin and eosin, the arrows represent pathological changes in tissue: (a) control groups: normal tissue $\times 200$; (b) H$_2$O$_2$ group: dilatation in the central vein $\times 200$; (c) H$_2$O$_2$ group: binucleation $\times 400$; (d) H$_2$O$_2$ group: inflammation $\times 400$; (e) H$_2$O$_2$ + Argan: dilatation in the central vein $\times 100$; (f) H$_2$O$_2$ + Argan + *Syzygium aromaticum* essential oil group: dilatation in the central vein $\times 200$.

![Figure 6](image6.png)

**Figure 6.** Histopathological evaluation of the kidney of the control and stressed groups, samples were stained with hematoxylin and eosin, the arrows represent pathological changes in tissue: (a) control groups: normal tissue $\times 200$; (b) H$_2$O$_2$ group: congestion $\times 200$; (c) H$_2$O$_2$ + Argan group: congestion $\times 200$; (d) H$_2$O$_2$ + Argan + *Syzygium aromaticum* essential oil group: normal tissue $\times 100$. 

The results of this study showed that Argan oil and *Syzygium aromaticum* essential oil have a protective effect on H$_2$O$_2$-induced biochemical changes and histopathological injury in kidney, liver, and spleen.
3. Discussion

The results of this study showed that Argan oil and *Syzygium aromaticum* essential oil has a protective effect on H$_2$O$_2$-induced biochemical changes and histopathological injury in kidney, liver and brain. The results demonstrated that *Syzygium aromaticum* essential oil has more antioxidant content than Argan oil. H$_2$O$_2$ causes significant increase in the lipid parameters, liver enzymes, and blood urea, significant increase in the liver and kidney weight, insignificant increase in the serum creatinine and significant decrease in the total protein and albumin. These H$_2$O$_2$-induced biochemical changes have been alleviated with use of *Syzygium aromaticum* essential oil or Argan oil.

The chemical composition of *Syzygium aromaticum* essential oil showed that eugenol and eugenol acetate were the main components, that was in agreement with other studies [31,32]. The antioxidant content in *Syzygium aromaticum* essential oil is higher than that found in Argan oil. Therefore *Syzygium aromaticum* essential oil might be more powerful as an antioxidant than Argan oil, and this property most likely due to the chemical composition of *Syzygium aromaticum* essential oil, which is rich in eugenol (87.03%) with a potent antioxidant activity [33,34].

The in vivo study demonstrated that H$_2$O$_2$ given in the drinking water (0.5%) causes significant increase in AST and ALT, decreases total protein and albumin, and an elevation of blood urea levels [35]. Another study showed that administration of H$_2$O$_2$ (0.1%) in drinking water in rats for 25 weeks induced an increase in the malondialdehyde levels, catalase activity, superoxide dismutase and glutathione peroxidase in organs [36].

In the present study daily administration of (1%) of H$_2$O$_2$ by gavage significantly increased the levels of LDH, ALT, AST, however, rats receiving H$_2$O$_2$ with Argan oil or *Syzygium aromaticum* essential oil emulsified in Argan oil had lower levels of LDH, ALT and AST in comparison with H$_2$O$_2$ groups. The increase of ALT and AST is an index of liver damage and alterations of liver function due to the release of these enzymes into the bloodstream from the cytosol [37,38].

The results also showed that H$_2$O$_2$ causes a decrease in albumin and total protein levels, but in the treated groups there is a total protection against the diminution of albumin in the group which receives the Argan oil alone and in the group, that receives Argan oil with *Syzygium aromaticum* essential oil. However, the protection against the diminution of total protein is better with Argan oil supplemented with *Syzygium aromaticum* essential oil than Argan oil alone. The decrease in albumin levels may be due to inflammation or liver failure [39].

Blood electrolyte (Na$^+$, K$^+$, Cl$^-$) and creatinine levels were not changed significantly during the experiments, but blood urea concentration was significantly increased in the group receiving H$_2$O$_2$ alone. Blood urea is a waste product of protein metabolism, synthesized in the liver and excreted by the kidney. Therefore, high blood urea could be due to renal damage which was not evident with stable and normal plasma creatinine. Furthermore, the elevated liver enzymes indicate abnormal liver function where low blood urea is expected. Therefore, mechanism of elevated blood urea with the use of H$_2$O$_2$ needs further experiments.

Regarding the lipid profile the results showed that H$_2$O$_2$ causes dyslipidemia with a decrease in the level of HDL-C and an increase in the levels of TC, LDL-C, and VLDL, whereas other groups, which received H$_2$O$_2$ combined with Argan oil or with Argan oil and *Syzygium aromaticum* essential oil, did not show similar dyslipidemia. It is well known that oxidative stress can induce lipid metabolism disorder and lipid peroxidation and this complication can cause many diseases such as cardiovascular diseases [5].

Regarding the organ weight the results showed that H$_2$O$_2$ significantly increases the liver and kidney weight and relative weights while it decreases the brain weight and the brain relative weight. This was also observed with the co-administration of H$_2$O$_2$ with Argan oil. However, co-administration with the *Syzygium aromaticum* essential oil and Argan oil did not affect the relative weight of the kidney. The changes in the weights and relative weights of the organs may be due to histopathological changes caused by H$_2$O$_2$. 
Interestingly, the present data showed that H$_2$O$_2$ induces histopathological changes in the liver, brain and kidney that include dilatation in the central vein, binucleation and inflammation in the liver, congestion in the kidney, and congestion, and hemorrhage in the brain tissue. In the liver, the histopathological changed accompanied by elevation of liver enzymes. The brain is known as the most sensitive organ to oxidative stress because of its high oxygen consumption and low antioxidant content [40]. The results are in agreement with a recent study reporting that oxidative stress causes congestion and cerebral hemorrhage [41].

The overproduction of free radicals following the gavage of rats by H$_2$O$_2$ is most likely the main cause of the histological and biochemical changes, which leads to an imbalance between the oxidant/antioxidant ratio.

In conclusion, the study shows that Argan oil and especially the mixture of Argan oil with Syzygium aromaticum essential oil can reduce the oxidative stress that is caused by H$_2$O$_2$. This protection is obviously due to the bioactive molecules and antioxidants such as eugenol in clove essential oil and vanillic acid, syringic acid, vitamin E and ferulic acid in Argan oil [42,43]. Further studies are needed to identify and characterize the most active materials in Argan oil and Syzygium aromaticum essential oil that might be suitable to be tested in clinical setting.

4. Materials and Methods

4.1. Argan Oil

The virgin Argan oil used in this study was obtained by mechanical press extraction from Agadir city, south west of Morocco, and was preserved at 4 °C in the dark container. In order to investigate the antioxidant effect of this oil, an extraction of phenolics compounds was used. Briefly, 10 g of the Argan oil was dissolved in 5 mL of n-hexane then extracted by liquid-liquid extraction with 10 mL of methanol/water (v/v, 60/40). The aliquot of the methanolic extract was preserved for the antioxidant activity testing [44].

4.2. Essential Oil Extraction

A total of 100 g clove was subjected to hydro-distillation for 3 h with 600 mL distilled water using a Clevenger-type apparatus modified: the hydrosol was collected in a separator funnel (1 L) so that the heavy essential oil was decanted to the bottom of the flask and collected. Another funnel of distilled water was used to add water to the flask containing the plant material during boiling. The essential oil obtained was collected and dried over anhydrous sodium sulfate and stored in a refrigerator at 4–5 °C prior to analysis. The yield based on dried weight of the sample was calculated.

4.3. Characterization and Chemical Composition of Syzygium aromaticum Essential Oil

4.3.1. Gas Chromatography Analysis

The isolated oil was diluted with hexane (dilution ratio 1:10), and 1 mL was sampled for the gas chromatographic analysis. Trace gas chromatograph (GC) (ULTRA S/N 20062969, Thermo Fischer, Villebon-sur-Yvette, France) that is equipped with HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, film thickness 0.25 mm) was used. Operating conditions: oven temperature program from 50 °C (2 min) to 280 °C at 5 °C/min and the final temperature kept for 10 min; 2 “split mode” ratio 1:20; carrier gas Azoth (N), flow rate 1 mL/min; temperature of injector and detector (flame ionization detector) were fixed at 250 °C and 280 °C, respectively.

4.3.2. Gas Chromatography–Mass Spectrometry (GC–MS)

The analysis of the volatile constituents was run on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; Polaris QS/N 210729), using an HP-5MS non polar fused silica capillary column (60 m × 0.32 mm, 0.25 mm film
thickness). The operating condition of GC oven temperature was maintained as: initial temperature 40 °C for 2 min, programmed rate 2 °C/min up to final temperature 260 °C with isotherm for 10 min; and injector temperature 250 °C. The carrier gas was helium, flow rate 1 mL/min. The samples were run in hexane with a dilution ratio of 1:10. The volume of injected specimen was 1mL of diluted oil, splitless injection technique; ionization energy 70 eV, in the electronic ionization mode; ion source temperature 200 °C, scan mass range of m/z 40–650 and interface line temperature 300 °C. The component identification was made by determination of their retention indices (KI) relative to those of a homologous series of n-alkanes (C₈–C₂₀) (Fluka, Buchs/sg, Buchs, Switzerland) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0, Gaithersburg, MD, USA) and the bibliography [45].

4.4. In Vitro Antioxidant Activities of Argan Oil and Syzygium aromaticum Essential Oil

4.4.1. Total Phenolic Content

The determination of the content of phenolic compounds was made by Folin–Ciocalteau method. Gallic acid was used as a reference. Syzygium aromaticum essential oil (50 µL) prepared in ethanol or Argan oil (100 µL) were mixed with 500 µL of Folin–Ciocalteau (0.2 N) reagent and 400 µL of sodium carbonate solution. The reaction mixture was incubated for 2 h in the dark, the absorbance was read at 760 nm, and the tests were made in triplicate [46,47].

4.4.2. Flavones and Flavonols

The content of flavones and flavonols was quantified as follows; 250 µL of Syzygium aromaticum essential oil prepared in ethanol or 250 µL of Argan oil were mixed with 250 µL of AlCl₃ solution, the reaction mixture was incubated for 1 h in the dark, the absorbance was read at 420 nm, the tests were made in triplicate, and quercetine was used as reference [48].

4.4.3. Total Flavonoids Content

To analyze the content of total flavonoids, 100 µL of Syzygium aromaticum essential oil prepared in ethanol or 100 µL of Argan oil were mixed with sodium nitrite (5%) and 150 µL of AlCl₃ solution 10%, 200 µL of NaOH (1%) 1M was added after 5 min, absorbance of the reaction mixture was measured at 510 nm, the tests were made in triplicate, and quercetine was used as reference [49].

4.4.4. Total Antioxidant Capacity (TAC)

The antioxidant capacity was evaluated by the phosphomolybdenum method. Syzygium aromaticum essential oil prepared in ethanol (25 µL) or Argan oil (25 µL) were mixed with 1 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After 90 min of incubation in a water bath at 95 °C the absorbance of the solution was measured at 695 nm against blank, the tests were made in triplicate, and quercetine was used as reference [50].

4.5. Experimental Animals

Thirty-six wistar rats (body weight 200 ± 20.18 g) were used for the experiment. The animals were housed in a standard environmental condition (25 ± 1 °C, 55 ± 5% humidity and 12 h/12 h light/dark cycle) and fed with rodent rats and free access to water. Experiments were conducted in accordance with the internationally accepted standard guidelines for the use of animals, and the protocol was approved by the institutional committee on animal care following the French Technical Specifications for the Production, Care and Use of the Laboratory and approval from the Ethical committee at Faculty of Sciences, Fez, Morocco was obtained.
4.6. Experimental Design

To evaluate the protective effect of Argan oil or *Syzygium aromaticum* essential oil prepared in Argan oil, the rats were randomly divided into six groups: Group 1: received (10 mL/kg/bw) of distilled water, Group 2: received *Syzygium aromaticum* essential oil prepared in Argan oil (100 mg/kg/bw), Group 3: received Argan oil (10 mL/kg/bw), group 4: received 1% of H$_2$O$_2$ (10 mL/kg/bw) and (10 mL/kg/bw) of distilled water, Group 5: received 1% of H$_2$O$_2$ (10 mL/kg/bw) and *Syzygium aromaticum* essential oil prepared in Argan oil (100 mg/kg/bw), and Group 6: received H$_2$O$_2$ (10 mL/kg/bw) and Argan oil (10 mL/kg/bw). The interventions were delivered daily by gavage for 21 days. Blood sample was collected from each rat on day 21 and body weight was measured. The kidney, brain, and liver of each rat were removed, weighted and were immediately fixed in formalin solution at (10%). The dose of Argan oil and *Syzygium aromaticum* essential oil were similar to the doses used elsewhere in rats [51,52].

4.7. Blood Analysis

After 3 weeks of treatment blood samples are withdrawn from each rat’s heart under anesthesia for analysis of lactate dehydrogenase (LDH); aspartate aminotransferase (AST); alanine transaminase (ALT); chloride; sodium; potassium; total cholesterol; triglycerides (TG); low density lipoprotein (LDL-C); high density lipoprotein (HDL-C); very low-density lipoprotein (VLDL); creatinine; blood urea; albumin; and total protein.

4.8. Histopathological Study

The study was conducted at Pathology Laboratory, University Hospital of Fez. After fixing the organs in the formalin solution (10%) for 48 h, the tissue samples were dehydrated in a series of increasing concentration of ethanol and clarified in toluene, then included in the paraffin. Sections of (5–6 mm) were prepared using a rotating microtome and stained with hematoxylin and eosin for observation under light microscope.

4.9. Statistical Analysis

Statistical analysis was carried out using GraphPad Software (San Diego, CA, USA) and data were represented as mean ± SD. ANOVA was performed and followed by Tukey’s multiple comparison tests. Student $t$-test was used to compare between two means. Throughout the analysis, $p < 0.05$ was considered significant.

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Abbreviations

| Acronym | Definition |
|---------|------------|
| ROS     | Reactive oxygen species |
| RNS     | reactive nitrogen species |
| GC/MS   | Gas chromatography–mass spectrometry |
| DPPH    | 1,1-diphenyl-2-picrylhydrazyl |
| TAC     | Total antioxidant capacity |
LDH  Lactate dehydrogenase
ALT  Alanine aminotransferase
AST  Aspartate aminotransferase
TC  Total cholesterol
TG  Triglycerides
LDL-C  Low Density Lipoprotein
HDL-C  High Density Lipoprotein-cholesterol
VLDL  Very Low Density Lipoprotein
BHT  Butylated hydroxytoluene
H & E  hematoxylin and eosin

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