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RESEARCH ARTICLE

COMPARATIVE STUDY OF THE ANTIOXIDANT PROPERTIES OF ESSENTIAL OILS OF *THYMUS VULGARIS* AND *OCIMUM GRATISSIMUM*, TWO PLANTS OF THE LAMIACEAE FAMILY

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ABSTRACT

The present work aim is to evaluate the antioxidant properties of the essential oils extracted from *Thymus vulgaris* and *Ocimum gratissimum* in order to promote their use in the food, cosmetic and pharmaceutical industries. It is about to establish a link between the chemical composition of these oils and their antioxidant properties. The composition of the essential oils was determined by gas chromatography. The antioxidant powers are evaluated using the reducing power of the ferrous ion and the trapping power of the radical DPPH°. The results show that both essential oils have similar chemical profiles. In fact, apart from α -pinene (8.80%) and α -selinene (5.50%) which are absent in the major compounds of the essential oil of *Thymus vulgaris*, and carvacrol (2.79%) absent in those of the essential oil of *Ocimum gratissimum*, all the other major constituents are present in the two essential oils with relatively variable contents. The IC₅₀ values, obtained using the DPPH method, prove that the oil of *Thymus vulgaris* is more antioxidant with 481.70 ppm than that of *Ocimum gratissimum* with 822.37 ppm. In addition, the results of the FRAP test show that the free radical activity (in Eq of Fe²⁺) of the oil of *Thymus vulgaris*, ie 603.50 ppm, is greater than that of the oil of *Ocimum gratissimum*, ie 513.50 ppm. The antioxidant activities presented by these oils could be attributed to the presence of their phenolic compounds. Used as food additives, these essential oils can thus contribute to reduce the risk of occurrence of certain diseases related to oxidative stress.

INTRODUCTION

Oxidative metabolism is essential for cell survival. Unfortunately it leads to the production of free radicals and other reactive species capable of creating oxidative damage (Koppenol, 2001). In the body, natural various causes are at the origin of the production of free radicals. Among them, oxidative respiration during which a small part of the oxygen escapes to the reduction in water and is responsible for the production of free radicals in the respiratory chain. In addition, during the antibacterial battle defense that accompanies any infection, leukocytes produce free radicals (Pincemail et al., 2002; Valko et al., 2007). However, the exogenous production of free radicals is becoming increasingly important. Indeed, living beings are exposed daily to all kinds of pollutants, which are at the origin of the production of free radicals once present in the organism (Mélila et al., 2013). Pollution sources include UV electromagnetic radiation, some transition metals, cigarette smoke, alcohol ingestion, pesticides and even some drugs (especially anti-cancer drugs such as anthracyclines) which

radical species would be responsible for their mode of action (Rolland, 2004 ; Pastre, 2005 ; Laguerre, 2007 ; Milane, 2014). To become stable, the free radicals attack other biological molecules leading to the creation of other free radicals which thus trigger chain reactions that have the disadvantage of modifying cells functional properties. Unfortunately, when the phenomenon grows, it probably causes certain chronic diseases (such as cardiovascular diseases, cancers, neurodegenerative diseases, etc.) and aging. These damages are caused by the attack of free radicals on various biomolecules, in particular proteins, lipids and DNA, irreparably resulting in the degradation cells death (Moon and Shibamoto, 2009). Even if all aerobic organisms including those of humans have an antioxidant defense system to regulate the formation of free radicals, this defense mechanism may not in some cases be effective enough. As result, the exogenous supply of antioxidant compounds becomes indispensable. Studies have shown that there is a correlation between a rich diet in antioxidants and the reverse incidence of diseases associated with oxidative stress (Pastre, 2005). The antioxidants (AO) are

compounds that help to prevent many oxidation reactions caused by free radicals. Their intervention is beneficial to ensure a safe health for living beings, as they can prevent cells damage. The free radicals can also cause lipid peroxidation in foods and accelerate their deterioration. However, the discovery of the inhibition of lipid peroxidation by phenolic compounds has led to the synthesis of some molecules for the food industry. Thus synthetic molecules such as butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) and propyl gallate (GP) have made their appearance as food preservatives (Balasundram *et al.*, 2006). However, some physical properties (volatility and instability) and toxicological (side effects) of BHA and BHT motivate for some time their prohibition. In addition, consumers are losing interest in foods that are preserved with synthetic chemicals (Odukoya *et al.*, 2005). Therefore, it becomes essential to find a solution to the conservation of food by preserving the quality and ensuring the safety of consumers.

Thus, in recent years, to preserve food for good health of consumers, plant extracts have become one of the major solutions with a consequent intake of antioxidants. Studies have shown that phenolic compounds in plants are able to prevent oxidative damage by trapping free radicals (Sökmen *et al.*, 2012) which are in various reactive forms of oxygen, such as the superoxide anion ($O_2^{\bullet-}$), the hydroxyl radical (OH^{\bullet}), the peroxy radical (ROO^{\bullet}), non-radical species including singlet oxygen (1O_2), hydrogen peroxide (H_2O_2) and some transition metals like the Fe^{2+} and the Cu^{2+} , acting as catalysts in the formation of the hydroxyl radical (Haleng *et al.*, 2007). Many polyphenols from plant such as vitamin E (α -tocopherol), flavonoids and flavones, carotenoids and vitamin C are bioantioxidants available. Some of them like β -carotene and lycopene are used as dyes. However, essential oils also start having a lot of interest as a source of potentially bioactive natural molecules with antioxidant capacity. Therefore, they are increasingly being studied for their possible use as alternatives in the treatment of certain infectious diseases and for the protection of foods against oxidative reactions (Haleng *et al.*, 2007). Lamiaceae family is the most widely used one as a source of spices and extracts with strong antibacterial and antioxidant powers (Viuda-Martos *et al.*, 2010). This family includes herbaceous plants, mostly aromatic, partially woody, forming shrubs (Evenamede, 2009). Currently, there is the question of the effectiveness of the molecules contained in the essential oils of Lamiaceae and the necessary tools for their possible studies. Many of tests have been developed in the literature to evaluate the ability of an antioxidant to brake or even better to stop or block the oxidation process. Essentially, the DPPH $^{\circ}$ radical reduction test, the ORAC (Oxygen Radical Absorbance Capacity) test, the Ferric Reducing Antioxidant Power (FRAP) test, and the Trolox Equivalent Antioxidant Capacity (TEAC) test are instructive (Agbodan *et al.*, 2014). The present study aims to study the antioxidant properties of *Thymus vulgaris*' essential oil compared with that of *Ocimum gratissimum* by the DPPH $^{\circ}$ radical reduction method and the FRAP test.

MATERIAL AND METHODS

Plant material: The essential oils tested in this study are extracted from two vegetables (*Thymus vulgaris* and *Ocimum gratissimum*) from the Lamiaceae family of Togolese flora, at the Laboratory of Plants Extracts and Natural Aromas by steam distillation. *Thymus vulgaris* is grown at Ahlon-Denou (Togo);

whereas *Ocimum gratissimum* is grown at the Lomé Agricultural Experimentation Station at the University of Lomé (Togo).

Essential oils extraction: The essential oils, obtained from these vegetables, were extracted within two hours term by steam distillation at the Laboratory of vegetables Extracts and Natural Aromas, University of Lomé (Togo).

Determination of the extraction yield and measurement of the refractive index: The extraction yield of each essential oil was determined by dividing the mass of the extracted essential oil by the mass of the dry biomass and its refractive index is measured using a refracto meter type Nippon Optical Works (NOW)/CO., LTD. Tokyo-Japan.

Analysis of the chemical composition of the essential oil of *Thymus vulgaris*: The chemical composition of *Thymus vulgaris* essential oil, compared to the one of *Ocimum gratissimum* (Agbodan *et al.*, 2015) is analyzed using an HP 5890 gas chromatograph (GC), equipped with a flame ionization detector (FID), an injector, and two types of columns: one apolar and the other polar. The detector flame is maintained by a Hydrogen/Air mixture, with flow rates of 30 and 300 mL/min, respectively. GC/MS analysis is also made using Agilent 5973 equipment with an HP $_1$ (MS 60m x 0.25mm x 0.25 μ m) apolar column at an initial temperature of 60 $^{\circ}C$ isothermal for 10 minutes, then at a final temperature of 300 $^{\circ}C$ for 20 min, with a gradient of 2 $^{\circ}C$ /minute. The carrier gas (helium) flow rate was set at 1 mL/min. The mass spectrometer detector is of HED/EM type (High Energy Dynode/Electron Multiplier 0-3000 V) with an energy of 70 eV; the other parameters remained identical.

Evaluation of the antioxidant activity of essential oils: The antioxidant capacity of the essential oils was evaluated in vitro by two spectrophotometric methods, namely: the measurement of the reducing power of the ferrous ion and the measurement of the trapping power of the radical DPPH $^{\circ}$.

Measurement of the trapping power of the radical DPPH $^{\circ}$: This test is based on the measurement of the antioxidant trapping capacity by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH $^{\circ}$). This radical is able to capture a hydrogen atom or an electron (Tirzitis and Bartosz, 2010) in order become to a stable molecule. The inhibitory activity of the DPPH $^{\circ}$ radical is evaluated by the decrease of the absorbance at 593 nm by its reduction in the presence of an antioxidant (AH), responsible for the loss of the dark purple color of the DPPH (Molyneux, 2004; Villaño *et al.*, 2007), according to the reaction illustrated in Figure 1.

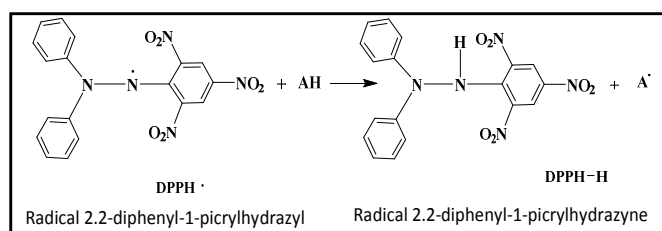


Figure 1. Reaction mechanism of DPPH radical reduction (Pyrzynska *et al.*, 2013)

Measurement of the reducing power of ferrous ion: The reducing power is the ability of antioxidants present in an

electron-donating solution (Li *et al.*, 2009) to reduce a ferric ion analogue (Tripyridyltriazine TPTZ- F_e^{3+} complex) to ferrous ion (TPTZ- F_e^{2+}) in a medium acidified with trichloroacetic acid (Luqman *et al.*, 2012). By adding F_eCl_3 into the mixture, a specific blue-colored complex is formed. During this oxidation-reduction reaction, the reduced form results in the change from yellow to blue (Chung *et al.*, 2006). The increasing in absorbance then corresponds to an increasing in the reducing power of the sample tested.

Preparation of reagents and essential oils to be tested: The DPPH solution, with 100 μ M concentration is prepared by dissolving 10 mg of DPPH in 254 mL of pure methanol. To avoid possible degradation, the essential oils prepared and FRAP reagent samples were stored in a refrigerator at 4 °C. But before use, they were taken out of the refrigerator and kept in the laboratory at room temperature and protected from light. However, for each test, the DPPH reagent was freshly prepared because of its high instability. At 20 mg of each essential oil sample introduced into a 20 mL volumetric flask, pure methanol was added up to the mark in order to obtain a concentration of 1000 ppm. From these stock solutions, dilutions were made using Eppendorf automatic pipettes (Agbodan *et al.*, 2014).

The reagent of the FRAP test was prepared according to the protocol described by Agbodan *et al.* (2014) which consists in mixing three solutions: a buffer solution with acid pH equal to = 3.5 (50 mL), a solution of 2,4,6-tripyridyl-s-triazine (TPTZ) (5 mL) and an iron chloride solution III (5 mL). The buffer solution (pH = 3.5) is prepared by introducing 0.310 g of $[CH_3COONa, 3H_2O]$ into a 100 mL flask; then by adding 1.6 mL of pure acetic acid, the previous mixture was supplemented with distilled water to the mark. The TPTZ solution was prepared by dissolving 156 mg of this compound into 50 mL of a hydrochloric acid solution (40 mM HCl). The solution of iron chloride III (20 mM) was obtained by dissolving 27 mg of $[F_eCl_3, 6H_2O]$ into 50 mL flask and supplementing with distilled water. The iron sulphate II solution, used as a reference for carrying out the FRAP test, was prepared by dissolving 27 mg of $F_eSO_4, 7H_2O$ into 50 mL of pure methanol. This stock solution of iron sulfate II (2000 μ M) was diluted to obtain different concentrations of F_e^{2+} ions to establish the calibration curve.

Optical density (OD) measurement

FRAP test case: A volume of 1.5 mL of the FRAP reagent was taken from a test tube to which 50 μ L of essential oil sample and 150 μ L distilled water were added. The mixture was vigorously vortexed and after 5 min of incubation in the dark, before reading the OD using a DMS 300 UV-V spectrophotometer, 593 nm wave. For each test, the sample was prepared in triplicate to determine the average and standard deviation of the DO measurement. The absorbance of previously prepared TPTZ- F_e^{2+} complex was used as a reference. In place of the sample, 50 μ L of the iron sulphate solution II with concentrations between 0 and 2000 mM are used. The OD measurements are used to plot a calibration curve whose equation is used to determine the F_e^{2+} ion equivalent for each sample of essential oil tested (Benzie *et al.*, 1999).

DPPH test case: A volume of 1.5 mL of the DPPH solution was taken from a test tube, to which was added 0.25 mL of an

essential oil solution. Quercetin solution was used as the reference antioxidant for this test. After vigorous stirring of the vortex mixture followed by incubation for 20 min in the dark, the optical density was read at the DMS 300 UV-V Spectrophotometer at the wavelength of 517 nm. For each test, the sample was prepared in triplicate to determine the mean and standard deviation of the DO measurement. For the control (blank) tube, the DO reading was made with 0.25 mL of methanol added to 1.5 mL of the DPPH solution, instead of 0.25 mL of the essential oil solution. The representative curve of DO versus essential oil concentration was plotted to determine the equation of the calibration curve using Excel software, and by extrapolation, to deduce the value of IC_{50} , that is, the antioxidant concentration needed to reduce the initial dyeing intensity of DPPH by 50%. Inhibition curves for IC_{50} values were plotted using the percentages of inhibitions calculated using Formula 1 (Mandal *et al.*, 2009; Hossain *et al.*, 2011).

$$\% \text{ Inhibition} = \frac{OD_{Blank} - OD_{Sample}}{OD_{Blank}} \times 100 \quad (\text{Formula 1})$$

The IC_{50} value obtained for each sample was compared with the one of quercetin, the reference antioxidant.

RESULTS

Extraction yield and refractive index of essential oils: The extraction yield and the refractive index of the two essential oils are shown in Table 1.

Chemical composition of essential oil of *Thymus vulgaris* in comparison with that of *Ocimum gratissimum*: After gas chromatography's analysis by, the majority of compounds contained in the essential oil of *Thymus vulgaris* are listed in Table 2, in comparison with those of the essential oil of *Ocimum gratissimum*.

Antioxidant capacities of essential oils in comparison with quercetin and F_e^{2+} ion: The measurement of the optical densities (OD) during the trapping of the DPPH $^{\circ}$ radical makes it possible to establish using the Excel software to trend a curve showing the variation of the OD according to the concentration of quercetin (Figure 2). Figures 4 and 5 present the trend curves showing the variation of the inhibition percentages as a function of the concentrations of the essential oils compared to the one of quercetin (Figure 3). From the Figures 3, 4 and 5, are calculated the IC_{50} (Table 3) of quercetin and essential oils studied. The percentages of essential oils trapping activities, compared the one of quercetin used as the reference antioxidant, are shown as the histograms of Figure 6. Figure 7 shows the trend curve showing the variation of the optical density as a function of the F_e^{2+} ion concentration. This curve was used to determine the reducing power of ferrous ion (F_e^{2+}) using the FRAP test. The equation of this trend curve and the OD values of the solutions of the essential oils were used to calculate the IC_{50} of the essential oils in F_e^{2+} ion equivalents (Table 3).

DISCUSSION

Extraction yield and refractive index of essential oils: In this study, the extraction yields of both essential oils are low and very close.

While the refractive index values of the two essential oils are in agreement with those provided by the literature and are in the existing standards (Haddouchi *et al.*, 2009).

Chemical composition of the essential oil of *Thymus vulgaris* in comparison with that of *Ocimum gratissimum*:

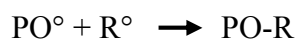
The major components of the essential oil of *Thymus vulgaris* are: thymol (29.49%), p-cymene (25.34%), γ -terpinene (10.77%), β -caryophyllene (3.2%), carvacrol (2.79%) and myrcene (1.93%), giving a total compound content of 73.52%. Previous studies by Agbodan *et al.* (2014) showed that *Ocimum gratissimum* contains as major constituents: thymol (21.32%), p-cymene (28.78%), α -pinene (8.8%), γ -terpinene (7.73%), α -selinene (5.50%), myrcene (4.75%) and β -caryophyllene (3.32%), ie a total content of 80.20% majority compounds. The comparison of the results (Table 2) shows that the two essential oils have similar chemical profiles in terms of the majority compounds, apart from α -pinene (8.80%) and α -selinene (5.50%) absent in the predominant compounds of the essential oil of *Thymus vulgaris*, then the carvacrol (2.79%) absent in the majority compounds of the essential oil of *Ocimum gratissimum*, all the other major constituents namely: thymol, para-cymene, γ -terpinene, β -caryophyllene and myrcene, are present in the both samples, but with more or less variable contents (Table 2).

Even if it happens sometimes that two plant species are source of essential oils of very close compositions, it is generally rare to notice that two plant species with essential oils with identical molecular constituents in their chemical compositions as it is noticed at the level of essential oils of *Thymus vulgaris* and *Ocimum gratissimum* studied here. Indeed, it is rather recognized that two species or subspecies very similar, belonging to the same genus, can give essential oils of different chemical compositions. Thus, for the same botanical species, there may be several chemical varieties or chemotypes that originate in slight differences in the biosynthetic pathways, resulting in the accumulation of different secondary metabolites (Afssaps, 2008). This phenomenon has been well studied for *Thymus vulgaris* L., for which at least seven (07) different chemotypes are distinguished according to the main constituent of essential oil (Pincemail *et al.*, 1999): alpha-terpineol, carvacrol, cineole, geraniol, sabinene hydrate, linalool and thymol. As result, it can be expected that the two types of essential oils can have substantially similar antioxidant properties. Antioxidant capacities of essential oils in comparison with quercetin and F_e^{2+} ion.

The shape of the curves (Figures 2, 3, 4 and 5) shows that the quercetin and the two essential oils studied here have antioxidant properties, independently of the two tests used to evaluate their antioxidant powers, since the DO varies linearly with the concentrations of essential oils. Thus, the IC_{50} values obtained using the DPPH method for quercetin, *Thymus vulgaris* and *Ocimum gratissimum*, namely 15.85 ppm, 481.70 ppm and 822.37 ppm respectively, prove that all the samples tested reduce the concentration of the radical DPPH $^{\circ}$ and then have anti-oxidant activities. These results show that the essential oil of *Thymus vulgaris* is more antioxidant than the essential oil of *Ocimum gratissimum*. But, it appears that the trapping capacities of the radical DPPH $^{\circ}$ by the essential oils are very weak compared to that of quercetin, since the results indicate that taking quercetin anti-radical activity as equal to 100%, those of essential oils of *Thymus vulgaris* and *Ocimum gratissimum* are respectively 3.29% and 1.93% (Figure 6).

This means that the anti-radical activity of quercetin is respectively 30.39 and 51.81 times that of the essential oils of *Thymus vulgaris* and *Ocimum gratissimum*. Concerning the FRAP test, the reduction of the ferric ion into ferrous ion is manifested by an appearance of the blue color and then an increase in the absorbance at 593 nm. The most reductive or antioxidant sample is then the one that induces a greater increase in absorbance after five minutes of incubation. The results of the FRAP test presented in Table 3, show that the free radical activity of the essential oil of *Thymus vulgaris*, ie 603.50 ppm (in Eq of F_e^{2+}), is also superior to the one of the essential oil of *Ocimum gratissimum*, ie 513.50 ppm (in Eq of F_e^{2+}). The results presented in this study corroborate those obtained by Agbodan *et al.* (2014) who, during their studies, obtained IC_{50} values equal to 20 ppm for quercetin and 875 ppm for the essential oil of *Ocimum gratissimum*, equivalent to F_e^{2+} equal to 515 $\mu\text{mol.L}^{-1}$ for essential oil of *Ocimum gratissimum* (Agbodan *et al.*, 2015). In this study, the anti-oxidant activities presented by the two essential oils would be attributed to the presence of phenolic compounds in their respective chemical compositions, with 57.62% and 50.10%, respectively, for essential oil of *Thymus vulgaris* and the *Ocimum gratissimum* one.

The molecular structures of the major compounds contained in these two essential oils (Table 4) indicate that thymol, carvacrol and p-cymene are the three phenolic compounds mostly involved in the DPPH $^{\circ}$ scavenging reactions and in the reduction of ferrous ion because the phenolic compounds have very appreciable anti-oxidant properties. These properties also justify their use in food and pharmaceutical products (Kouassi *et al.*, 2018). However, the presence of phenolic compounds alone can not justify the antioxidant properties of essential oils. Indeed, the presence of other compounds such as alcohols, aldehydes, monoterpenic ketones, phenylpropanes and monoterpenes could act by action synergy or by addition of effect. The results (Table 3) show that the essential oil of *Thymus vulgaris* is more antioxidant than that of *Ocimum gratissimum*. This is explained by the fact that the total content of phenolic compounds in the essential oil of *Thymus vulgaris*, 57.62%, is relatively higher than that of the essential oil of *Ocimum gratissimum*, ie 50.10%. Because of their low redox potential, polyphenols (Ar-OH) are able to rapidly reduce oxidative free radicals such as superoxide ($O_2^{\cdot-}$), peroxides (ROO^{\cdot}), alkoxy (RO^{\cdot}) and hydroxyl by hydrogen transfer according to the reaction mechanism of Figure 8. It is recognized that the antioxidant activity of phenolic compounds is due to their ability to trap free radicals to give the hydrogen atom and electron to chelate metal cations. The structure of the phenolic compounds is then the determining element of their antioxidant activity (Balasundram *et al.*, 2006). Phenoxide (PO^{\cdot}) intermediate radicals are relatively stable due to resonance and therefore, a new chain reaction is not easy to initiate (Dai and Mumper, 2010). Moreover, they can act with other free radicals according to the reaction:



The phenolic compounds have an ideal chemical structure for scavenging free radicals because they have hydroxyl phenolic groups which are capable of giving a hydrogen atom or an electron to the free radical resulting to the formation of an aromatic system stabilized by resonance (Dai and Mumper, 2010).

Table 1. Extraction yield and refractive index of the two essential oils

Samples of essential oils	Extraction yield	Refractive index at 20 °C	Standards
<i>Thymus vulgaris</i>	0,76%	1,4915	1,491 - 1,510
<i>Ocimum gratissimum</i>	0,60%	1,5422	1,475 - 1,501

Table 2. Comparison between the chemical composition of the majority compounds of *Thymus vulgaris* essential oil and the one of *Ocimum gratissimum*

Type of essential oil	Majority compounds identified by GC analysis	Contents (%)	References
<i>Thymus vulgaris</i>	Thymol	29,49	Our study
	p-cymene	25,34	
	γ -terpinene	10,77	
	β -caryophyllene	3,20	
	Carvacrol	2,79	
	Myrcene	1,93	
	Total (%)	73,52	
<i>Ocimum gratissimum</i>	Thymol	21,32	Agbodan et al. (2015)
	p-cymene	28,78	
	α -pinene	8,80	
	γ -terpinene	7,73	
	α -sélénine	5,50	
	Myrcene	4,75	
	Total (%)	80,20	

Table 3. Antioxidant power of essential oils tested in comparison with quercetin

Tests performed	Measured parameters	Samples		
		Quercetin	<i>Thymus vulgaris</i>	<i>Ocimum gratissimum</i>
DPPH	IC ₅₀ (ppm)	15,85	481,70	822,37
FRAP	Equivalent in Fe ²⁺ (μ M)	---	603,50	513,50

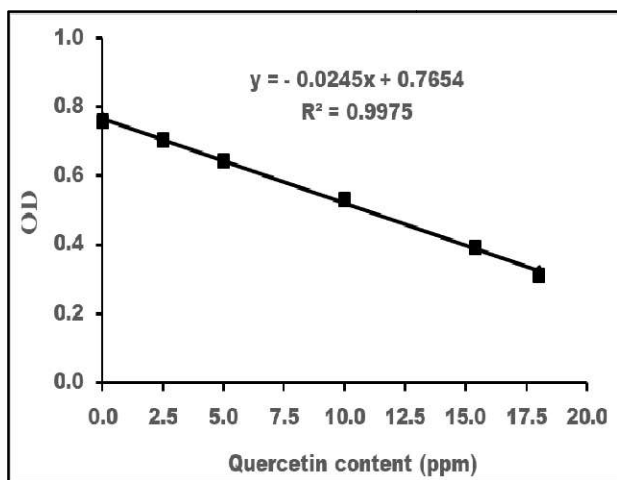


Figure 2. Trend curve showing the variation of OD in relation to quercetin concentration

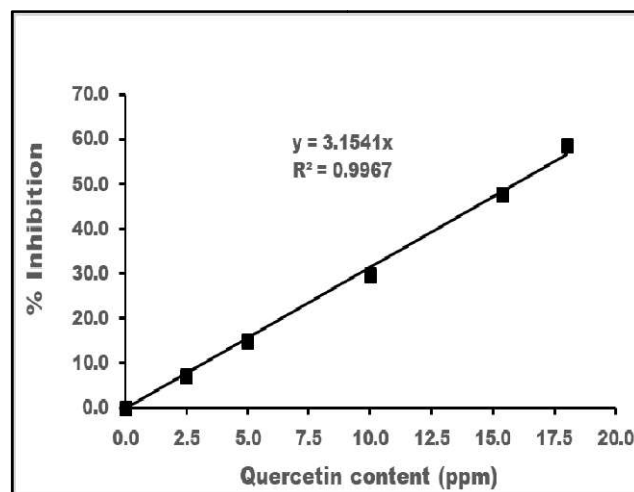
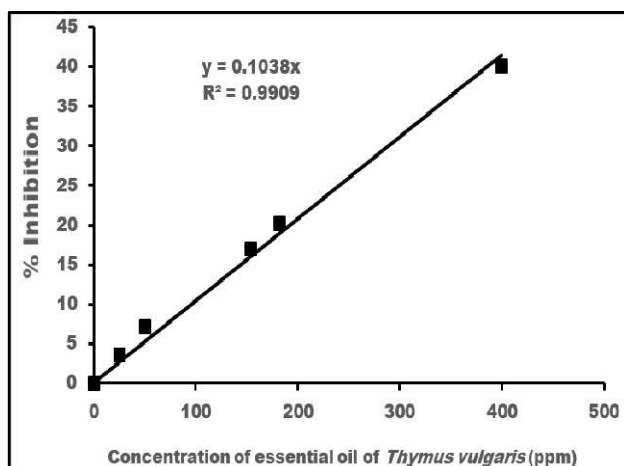
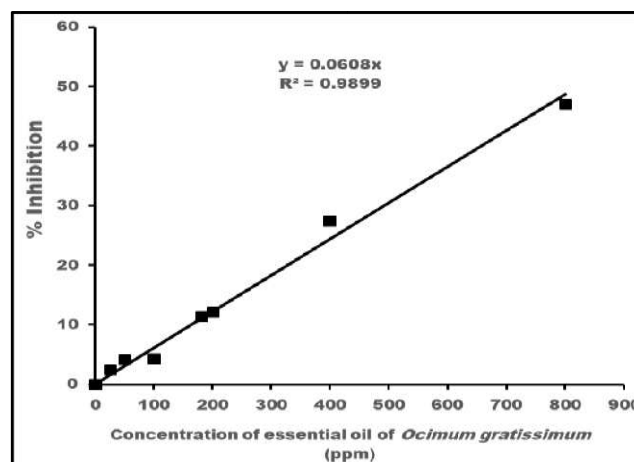


Figure 3. Trend curve showing the variation of DO in relation to quercetin concentration

Figure 4. Trend curve showing %Inhibition variation in relation to essential oil of *Thymus vulgaris* concentrationFigure 5. Trend curve showing %Inhibition variation in relation to essential oil of *Ocimum gratissimum* concentration

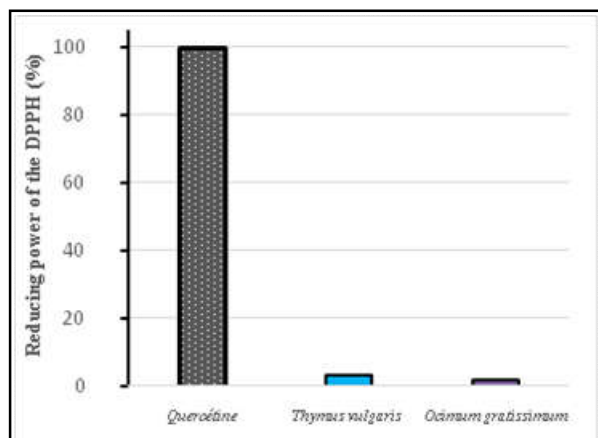


Figure 6. Percentage of DPPH sequestration activities of essential oils compared to quercetin

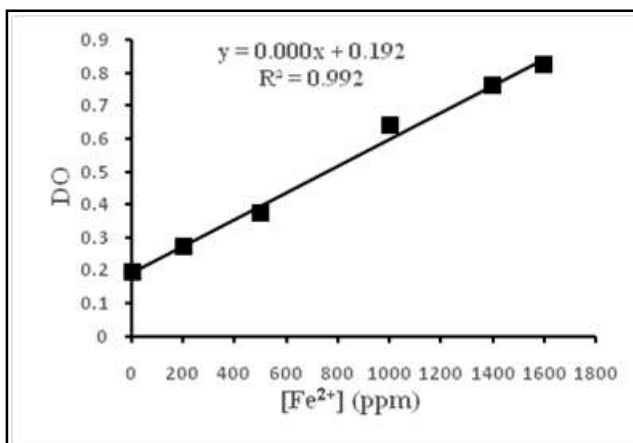


Figure 7. Trend curve showing optical density versus Fe²⁺ ion concentration

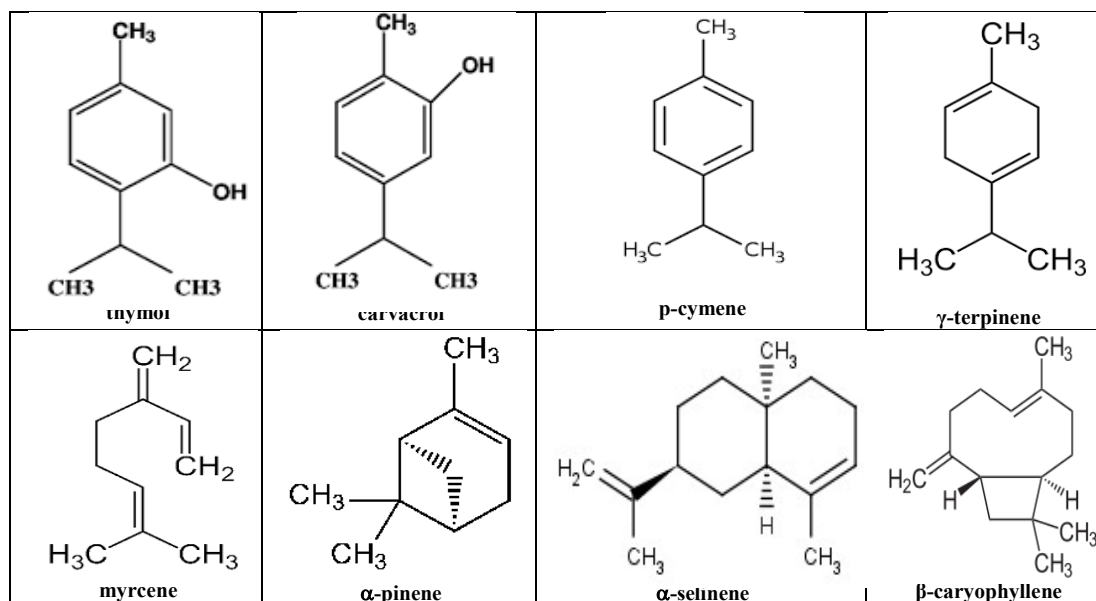


Table 4. Molecular Structures of majority compounds present in both essential oils

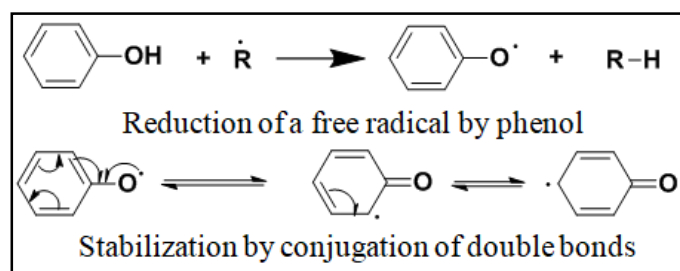


Figure 8. Reaction mechanism of reducing in phenolic compounds with free radicals: case of phenol (Rolland, 2004; Rowland et al., 2017)

Conclusion

The present study has demonstrated the antioxidant properties of essential oils *Thymus vulgaris* and *Ocimum gratissimum*, two plants of the Lamiaceae family, more used in traditional medicine in Togo. The two methods used to evaluate the antioxidant properties reveal that both essential oils possess interesting antioxidant properties. This biological property could be due to the presence of phenolic compounds, such as: thymol, carvacrol and p-cymene, mainly present in both essential oils. We can then consider developing standardized phytomedicines and nutraceuticals based on these essential oils

to help reduce the risk of occurrence of certain diseases related to oxidative stress. Since the use of synthesized molecules for the protection against oxidative damage is, in some cases, the cause of secondary effects, the use of natural molecules with antioxidant properties of less toxicity proves to be an important step alternative to the use of these synthetic molecules. However, in order to exploit the antioxidant potential of the essential oils tested, it is important to carry out additional studies to evaluate the antioxidant potential of these essential oils in vivo in order to determine the dose to which they do not pose risks of toxicity to mammals.

REFERENCES

- AFSSAPS. 2008. Agence française de sécurité sanitaire des produits de santé. Disponible sur <http://www.afssaps.sante.fr>; consulté le 27 mai 2019.
- Agbodan K. A., Dotse K., Koumaglo K. H. 2014. Activités antioxydantes des huiles essentielles de trois plantes aromatiques acclimatées au Togo. *Int. J. Biol. Chem. Sci.*, 8 (3): 1103-1110.
- Agbodan K. A., Dotse K., Koumaglo K. H. 2014. Activités antioxydantes des huiles essentielles de trois plantes aromatiques acclimatées au Togo. *Int. J. Biol. Chem. Sci.*, 8 (3): 1103-1110.
- Agbodan K. A., Dotse K., Koumaglo K. H., Saloufou K. I. 2015. Effets d'un acide fort sur la réaction de la DPPH avec deux huiles essentielles de deux plantes aromatiques acclimatées au Togo. *Int. J. Biol. Chem. Sci.*, 9(6): 2932-2939, 2015.
- Balasudram N., Sundram K., Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. *Food Chem.*, 99: 191-203.
- Chung Y., Chien C., Teng K., Chou S. 2006. Antioxidative and mutagenic properties of *Zanthoxylum ailanthoides* Sieb & zucc. *Food Chemistry*, 97: 418-425.
- Dai J., Mumper R. J. 2010. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. *Molecules*, 15 (10): 7313 – 7352.
- Evenamede K. S. 2009. Etude de la composition chimique des huiles essentielles d'*Ocimum canum*, de *Monodora myristica* et de quelques agrumes : plantes aromatiques du Togo. *Mémoire de de Diplôme d'Etude Approfondie (DEA) de chimie, Université de Lomé*, 77p.
- Haddouchi F., Lazouni H. A., Meziane A., Benmansour A. 2009. Etude physicochimique et microbiologique de l'huile essentielle de *Thymus fontanesii* Boiss & Reut. *Afrique SCIENCE*, 5 (2) : 246 – 259.
- Haleng J., Pincemail J., Defraigne J.O., Charlier C., Chapelle J. P. 2007. Le stress oxydant. *Rev Med Liege*, 62 (10) : 628-638.
- Hossain M. A. and Nagooru M. R. 2011. Biochemical profiling and total flavonoids contents of leaves crude extract of endemic medicinal plant *Corydalis terminalis* L. Kunth. *Pharmacognosy Journal*, 3 (24): 25-30.
- Koppenol W. H. 2001. 100 years of peroxyinitrite chemistry and 11 years of peroxyinitrite biochemistry. *Redox Rep.*, 6(6): 339-41.
- Kouassi E. K., Ouattara S., Seguin C., Fournel S., Frisch B. 2018. Etude de quelques propriétés biologiques de *Ocimum gratissimum* L., Une Lamiaceae Récoltée à Daloa (Côte d'Ivoire). *European Scientific Journal*, 14: 477-493.
- Laguerre M., López-giraldo L. J., Lecomte J., Pina M., Villeneuve P. 2007. Outils d'évaluation in vitro de la capacité antioxydante. *Oléagineux, Corps Gras, Lipides*, 14 (5) : 278-292.
- Li X., Wu X., Huang L. 2009. Correlation between antioxidant activities and phenolic contents of radix *Angelicae sinensis* (Danggui). *Molecules*, 14: 5349–5361.
- Luqman S., Srivastava S., Kumar R., Maurya A. K., and Chanda D. 2012. Experimental Assessment of *Moringa oleifera* Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using *In Vitro* and *In Vivo* Assays. *Evidence-Based Complementary and Alternative Medicine*, Article ID 519084, <http://dx.doi.org/10.1155/2012/519084>.
- Mandal P., Misra T.K., Ghosal M. 2009. Free radical scavenging activity and phytochemical analysis in the leaf and stem of *Dr. Imaria Diandra* Blume. *Int J Integr Biol*, 7 (2): 80-84.
- Mélila M., Poutouli W., Houndji B., Tchaou M., Pakoussi T., Awaga K. L., Bilabina I., Tozou P., Badanaro F., Abalikoka E.-Y., Gnandi K., Agbonon A., Agbèrè S., Tchabédji G., Sanni A., Amouzou K., Gueant J.-L. 2013. Oxidative stress in human due to metallic traces elements bioconcentration in three coastal villages near phosphate treatment factory in Togo. *Africa Journal of Food Science and Technology*, 4(6): 141 – 147.
- Milane H. 2004. La quercétine et ses dérivés: molécules à caractère pro-oxydantes ou capteurs de radicaux libres; études et applications thérapeutiques. *Thèse de doctorat, Université Louis Pasteur Strasbourg I*, 267p.
- Molyneux P. 2004. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarinn J. Sci. Technol.*, 26: 211–219.
- Moon J. K., Shibamoto T. 2009. Antioxidant assays for plant and food components. *J Agr*
- Odokoya A. O., Ilori O., Sofidiya M. O., Aniunoh O. A., Lawal B., Mansurat I., Olufunmilola A. 2005. Antioxidant activity of nigerian dietary spices. *EJAFChE*, 4 (6): 1086-1093.
- Pastre C. O. J. 2005. Intérêt de la supplémentation en antioxydants dans l'alimentation des carnivores domestiques. *Thèse de doctorat, Université Paul-Sabatier de Toulouse*, 117p.
- Pincemail J., Bonjean K., Cayeux K., Defraigne J. O. 2002. Mécanismes physiologiques de la défense anti-oxydante. Physiological action of antioxidant defences. *Nutrition clinique et métabolisme*, 16: 233-239.
- Pincemail J., Meurisse M., Limet R., Defraigne J. O. 1999. Espèces Oxygénées Activées, antioxydants et cancer. *Vaisseaux, cancer, poumons*, 4 (4) : 4p. Disponible sur <https://www.fabriceleu.com/wp-content/uploads/2018/05/cancer-article.pdf>.
- Pyrzynska K. and Pękal A. 2013. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Anal. Methods*, 5: 4288-4295.
- Rolland Y. 2004. Antioxydants naturels végétaux. *Oléagineux, Corps Gras, Lipides*, 11 (6): 419-424.
- Rowland I., Gibson G., Heinken A., Scott K., Swann J., Thiele I., Tuohy K. 2017. Gut microbiota functions: metabolism of nutrients and other food components. *European Journal of Nutrition*, 57 (1): 1-24.
- Sökmen, B. B., Aydın, S., & Kınalıoğlu, K. 2012. Antioxidant and antibacterial properties of a lichen species *Diploschistes scruposus* (Schreb.) Norman. *IUFS Journal of Biology*, 71 (1): 43 –51.
- Tirzitis G. and Bartosz G. 2010. Determination of antiradical and antioxidant activity: basic principles and new insights. *Acta Biochimica Polonica*, 57: 139–142.
- Valko M., Leibfritz D., Moncol J., Cronin M. T., Mazur M., Telser J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International journal of biochemistry & cell biology*, 39(1) : 44-84.
- Villaño D., Fernández-Pachóna M. S., Moyá M. L., Troncoso A. M., García-Parrilla M. C. 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, 71 (1): 230-235.
- Viuda-Martos M., Ruiz N. Y., Sánchez Z. E., Apata J., Fernández-López J., Pérez-Álvarez A. J. 2010. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. *FlavourFragr. J.*, 25 : 13–19.
- Wichtl M. Anton R. 2003. *Plantes thérapeutiques* 2^{ème} Edition, *Lavoisier Tec et Doc*, Paris.