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Biochemical Parameters and Antioxidant Activity of Fresh and Dry Ginger Rhizomes (*Zingiber officinale*). Comparative Study

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ABSTRACT

In Algeria, many plant resources, including ginger (*Zingiber officinale*), are experiencing a revival of interest in the dietary habits of local populations who associate them with their ancestral culinary and curative traditions. The aim of this study was to extract and characterize some physicochemical parameters of the essential oils (EO) of the fresh and dry ginger rhizomes and to evaluate, in-vitro, their antioxidant activity by scavenging the free radical DPPH. The extraction of the EO was realized by the hydrodistillation method.

Steam distillation of the EO from the fresh and dry ginger samples provided yields of 0.42 ± 0.017 and 1.55 ± 0.034 %, respectively. The evaluation of their physicochemical characteristics, in the same order, according to AFNOR standards made it possible to find the following results: refractive index: 1.4707 ± 0.001 and 1.4853 ± 0.0011 ; density 0.8748 ± 0.0005 and 0.8837 ± 0.0005 ; acid number: 1.4707 ± 0.0086 and 1.4853 ± 0.017 mg KOH / g; ester index: $15,732 \pm 0.24$ and $18,36 \pm 0,41$ mg KOH / g.

The evaluation of the antioxidant activity by the DPPH free radical scavenging method shows that the fresh rhizomes of *Zingiber officinale* EOs displayed significantly ($p < 0,01$) higher activity than the dried ones. Nevertheless, both Ginger forms EOs had lower activity than vitamin E ($p < 0,001$) which remains the best with $EC_{50} = 309,5$, namely (EO of fresh ginger, $EC_{50} = 723 \mu\text{g} / \text{ml}$; EO of dry ginger, $IC_{50} = 774 \mu\text{g} / \text{ml}$), all the statistical results of the parameters studied showed significant differences between the two types of ginger.

INTRODUCTION

Oxidative stress corresponds to an imbalance between the pro-oxidant and antioxidant systems. It is often responsible of the appearance of several diseases spanning cardiovascular, inflammatory syndromes, cancer and arteriosclerosis (Atamer, 2008). To avoid the consequences of oxidative stress, the body, in addition to the natural substances produced by it, needs antioxidant supplements to compensate for this imbalance and prevent or repair oxidative damage (Halliwell et Gutteridge, 2008).

Therefore, the use of antioxidants is remarkably increasing especially from medicinal plants that are a natural source of bioactive molecules providing promissory bioactive effects for human beings. They are also supposed to replace synthetic antioxidants which are being abandoned due to their side effects such as carcinogenicity (Kumaran and Karunakaran, 2007).

The bioactive molecules may contain free-radical scavengers, reducing agents and potential singlet oxygen quenchers (Bruneton, 2009). Among the well-known medicinal plants, Ginger (*Zingiber officinale*) is consumed worldwide as a spice and flavoring agent from ancient times (Gigon, 2012). This plant, in its fresh state, remained for a long time unknown to the Algerian consumer who used it only in its dried form. It is solely during the last two decades that fresh ginger benefited from a large interest in all the territory for its gustatory qualities as a spice and for its medicinal virtues.

Also, recent studies showed that the essential oils extracted from the rhizomes of this plant (fresh and dry) contained bioactive molecules also called phytochemicals such as sesquiterpenes, flavonoids and polyphenols that have healing properties that can block the action of reactive oxygen species and protect the body against oxidative damage and many other diseases (Bruneton, 2009).

The aim of this study was to investigate some physicochemical properties and to compare the in-vitro antioxidant activity of fresh and dry Ginger rhizome extract.

MATERIALS AND METHODS

Material:

1. Chemicals: Ethanol 95, Methanol, Vitamin C, Folin-ciocalteu reagent, Indigosulphonic acid, α - α diphenyl β picryl hydrazyl (DPPH), Riboflavin, Nitro Blue Tetrazolium (NBT) and Dimethyl Sulphoxide (DMSO), Potassium Hydroxide (KOH). All chemicals were purchased from Merck chemicals, all other reagents used

were of analytical grade.

2. Instruments: Analytical balance (TE214S, SARTORIUS), Water distiller « SCHOTT », UV Spectrophotometer (UVmini-1240 CE, SHIMADZU CORPORATION), Refractometer (OPTIKA), Pycnometer, Laboratory Centrifuge Machine (ROTOFIX 32A, HETTICH ZENTRIFIGEN).

3. Plant Material: The rhizome of *Zingiber officinale* which is imported from (China) was bought from Sidi-Bel-Abbes (Algeria) local market. It was formally identified by botanical specialists.

Methods:

1. Essential Oil Yields: According to the AFNOR standard (1986), the EO yield is defined as the ratio between the mass of EO collected after distillation and the mass of the plant material used, expressed as a percentage.

2. Sensory Analysis: In order to characterize the ginger EOs, it is necessary to study their organoleptic characteristics, mainly their appearance, color and smell through a panel of assessors.

3. Physicochemical Characteristics:

3.1 Chemical Indices:

3.1.1. The Acid Number (NF ISO 1242: 1999 (NF T 75-103)): The acid number of a fatty substance is the quantity of potassium hydroxide (KOH) in mg necessary to neutralize its free acidity. The free acid content of fats increases over time, so the acid number allows us to judge their state of deterioration.

3.1.2 Ester Index (AFNOR NF T 75-104: 1994): The ester index, E.I. is the number of milligrams of potassium hydroxide necessary to neutralize the acids released by the hydrolysis of esters contained in 1g of EO.

3.2 Physical Characteristics:

3.2.1 Relative Density at 20°C (AFNOR NF ISO 279: 1999): The density (density) is a physical quantity that characterizes the mass of a material per unit volume.

In definition, the relative density at 20 ° C of an EO is the ratio of the mass of a certain volume of oil at 20 ° C, and the mass

of an equal volume of distilled water at 20 ° C. Its symbol is d20/20

3.2.2 Refractive Index η : (AFNOR NF ISO 280: 1998): It is the ratio of the sine of the angle of incidence to the sine of the angle of refraction, when a ray of light of defined wavelength passes from air into the essential oil kept at a constant temperature.

3. Evaluation of the Antioxidant Activity of The Essential Oil of Ginger: The objective was to compare the antioxidant activity of the essential oil of the medicinal plant *Zingiber officinale* by the method of determination of the oxidation of the 2,2-diphenyl-1-picrylhydrazyl radical called DPPH.

3.1. Preparation of Standard Stock Solution of Vitamin E: Accurately weighed 10 mg of vitamin E was transferred to 10-mL amber colored volumetric flask. Dissolved and diluted up to the mark with methanol (100% v/v) to give stock solution of 1000 $\mu\text{g/mL}$ of vitamin E. It was prepared freshly and used immediately for the study. From the stock solution, different concentrations were prepared to evaluate antioxidant capacities: 100, 200, 400, 600, 800 and 1000 $\mu\text{g/mL}$.

3.2. DPPH Radical Scavenging Activity: Ginger EO and standard vitamin E solution (0.1 ml) of different concentrations were added to 3 ml of a 0.004% methanol solution of DPPH. An equal amount of

methanol and DPPH served as control. After 30 minutes of incubation in the dark, absorbance was recorded at 517 nm, and the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the EO/standard. The antioxidant activity of the oil was expressed as IC_{50} .

The IC_{50} value is defined as the concentration of extracts expressed in $\mu\text{g/mL}$ that inhibits the formation of DPPH radicals by 50%. All the tests were performed in triplicate and the graph was plotted with the average of three observations (Kumaran and Karunakaran, 2007).

4. Statistical Evaluation: Experimental results were Mean \pm SD of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value. Student's t-test was used for the comparison between two means for the possible significant interrelation.

Data were entered into Excel and analyzed using SPSS version 20 software and were considered statistically significant only when p value < 0.05.

RESULTS

1. Yield of Essential Oil:

After hydrodistillation, the recovered EOs were weighed. The resulting values are depicted in the following Fig. 1Fig. 1:

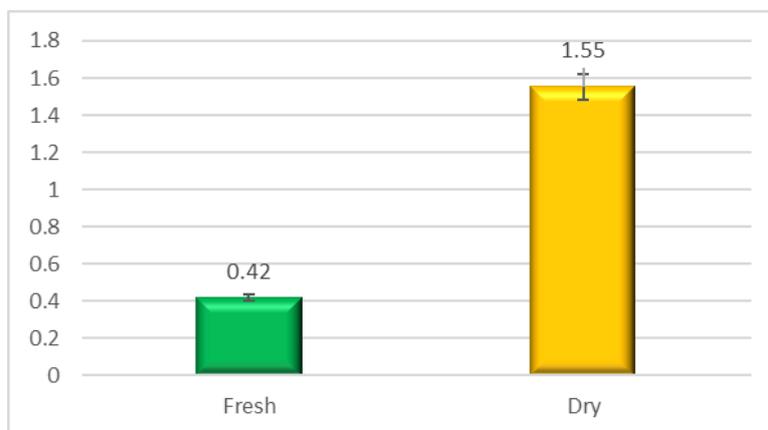


Fig. 1: Fresh and Dry Essential oil Ginger yields.

A significant difference ($p < 0,01$) was found between the two essential oils: $0,42 \pm 0,017$ and $1,55 \pm 0,034$ of fresh and dry ginger respectively. The proportion of water in the plant material was taken as a factor of variation of the extract content; this allows us to deduce that the effect of drying ginger increased the concentration of its essential oil by about 27.1%.

This difference could be explained according to (Kelen and Tepe, 2008). By the choice of the period of harvest which remains primordial in determining output and quality of the EO.

2. Analytical Study of the Extract of *Zingiber Officinale*:

The organoleptic and

physicochemical properties are means of verifying and controlling the quality of the EO. Our tests were performed according to a precise protocol in accordance with the standards set by the ISO. For the EO of *Zingiber officinale* it is the standard NF ISO 4731:2006 (NF T 75-212) which is in force.

2.1 Organoleptic properties of EO:

The organoleptic characteristics of an EO are necessary data for the assessment of the quality of the oil, both from a scientific and commercial point of view. Indeed, it is generally accepted that these characteristics can be correlated with the chemical composition. Our visual and olfactory perceptions of the essential oils obtained are reported in the Table 1.

Table 1: Organoleptic characteristics of fresh and dry ginger's EO obtained.

Essential oil of <i>Zingiber Officinale</i>	STATE	Organoleptic caractéristiques		
		Aspect	Color	Odor
	Fresh	Mobile and clear liquid	Very light yellow/ pale yellow	Characteristic, reminiscent of the rhizome of ginger
	Dry	Liquid	Dark yellow	Warm spicy

2. Physicochemical Characteristics:

The physicochemical characteristics of ginger EO were determined according to AFNOR standards.

2.1 Relative Density at 20°C:

The results of the density

measurements are presented as histograms in the Fig. 2. The density of fresh ginger EO is slightly lower than the one of dry ginger ($0,8748 \pm 0,0005$ and $0,8837 \pm 0,0005$) since it contains more monoterpenes (Jayashree *et al.*, 2014).

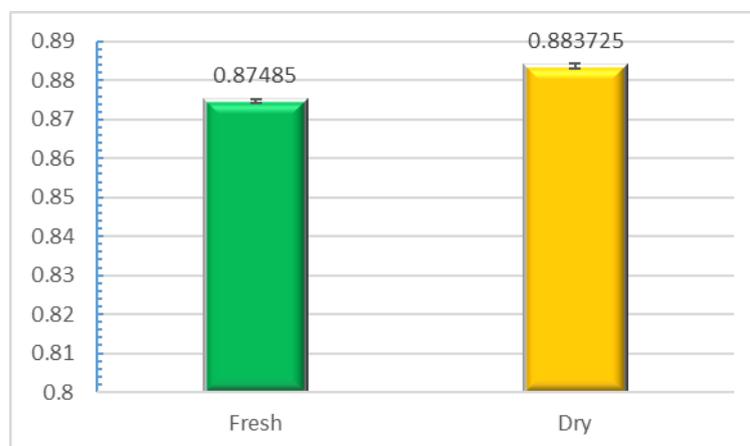


Fig. 2: Density of fresh and dry ginger's essential oils.

2.2 Index of Refraction:

The refractive index is the ratio between the speed of light in a vacuum and that in the medium considered. This ratio indicates the capacity of the EOs to reflect the light.

To calculate the refractive index, we use the following formula:
The temperature (t') is equal to 22°C and the

reading on the device gave the following results (Fig. 3). The refractive index of fresh ginger EO obtained is slightly lower than that obtained by dry ginger viz 1,4707±0,001 and 1,4853±0,0011. This may be due to the composition of the fractions contained, especially the volatile compounds (Boukhatem *et al.*, 2016).

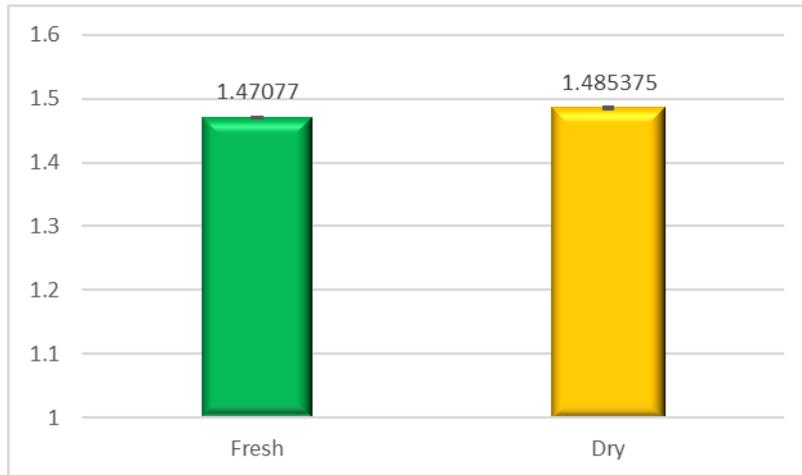


Fig. 3: Refractive index of fresh and dry ginger’s essential oils.

2.3 Acid Number:

The acid number (AN) results of the different ginger samples are shown in Fig. 4. The acid number gives an idea about the free acid content. From the results obtained (fresh ginger 1,4707±0,0086, dry ginger

1,4853±0,017) we can say that the acid value of fresh ginger EO is lower than that of dry ginger EO. Both Values are lower than 2, which is an indication of good conservation of the essence (i.e., low amount of free acids) (Kanko *et al.*, 2004).

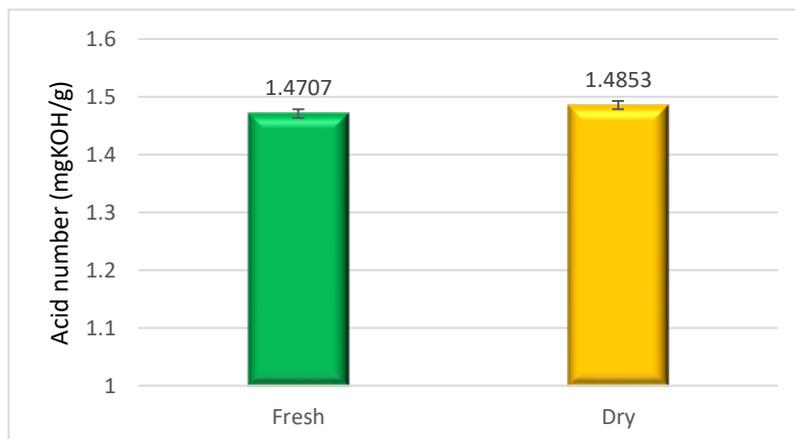


Fig. 4: Acid number of fresh and dry ginger’s essential oils (mg KOH/g).

2.4 Ester Index:

The results are presented on Fig. 5. The ester index of dry ginger EO ($18,36 \pm 0,41$) is slightly higher ($p < 0.01$) than

that obtained for fresh ginger EO ($15,732 \pm 0,24$). A high EI is a guarantee of the quality of a fraction (Kanko *et al.*, 2004).

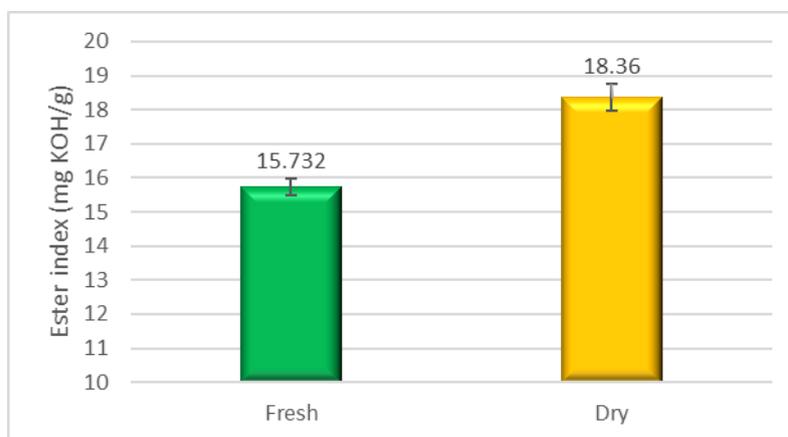


Fig. 5: Ester Index of fresh and dry ginger's essential oils.

3. DPPH Radical Scavenging Activity:

Results of the antioxidant activity of fresh and dry ginger EO on the DPPH radical compared to the standard antioxidant (vitamin E) were evaluated using a spectrophotometer by following the reduction of this radical which is accompanied by its passage from the violet color (DPPH-) to the yellow color (DPPH-H) measurable at 517 nm. The *antiradical capacity* is determined by decreased absorbance induced by antiradical substances (Majhenic *et al.*, 2007)

The results of the absorbance of different dilutions of reaction mixtures of all

samples are illustrated hereunder (Fig. 6). A reduction in the absorbance of DPPH in solution is observed with increasing the concentration of each EO sample.

Antioxidant activity is dependent on the mobility of the hydrogen atoms on the hydroxyl group of the phenolic compounds in the EO. In the presence of a DPPH-free radical, the H atom is transferred to this latter which turns it into a stable DPPH molecule. This causes a decrease in the concentration of the free radical and also the absorbance over the reaction time until the capacity of the antioxidant providing the hydrogen is exhausted (Villano *et al.*, 2007).

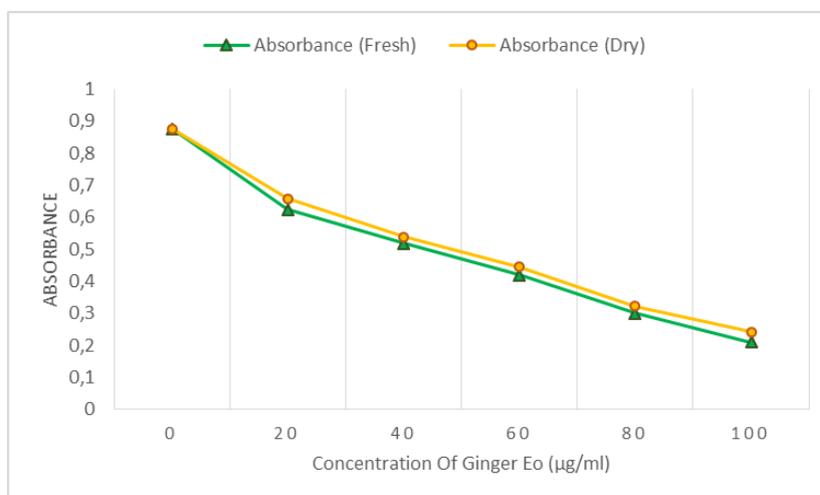


Fig. 6: Absorbance of DPPH decreasing by Essential Oil of fresh and dry ginger.

3.1 Determination of Percentage Inhibition (I%):

The measurements of the absorbance of DPPH after 30 minutes allowed the calculation of the percentage of inhibition (I%) of each dilution of the essential oil. It is calculated by applying the formula previously cited in the section "material and methods".

The results obtained in the DPPH radical measurement test of the two samples (Fresh and dry ginger, vitamin E) at various concentrations are represented in Fig. 7. Results showed that the percentage of free

radical inhibition increased with the increase of concentration for either vitamin E or both samples of ginger extract. It also revealed a significant ($p < 0.05$) difference of DPPH scavenging activity in both Ginger essential oils.

It is noted that the percentage of free radical inhibition for the extract is lower than that of vitamin E for all concentrations used. For a concentration of 1000 $\mu\text{g/ml}$, fresh ginger extract revealed a maximum percentage of inhibition of 65.94 % and dry ginger extract 62.29% versus a percentage of inhibition for vitamin E of 90.94 % DPPH.

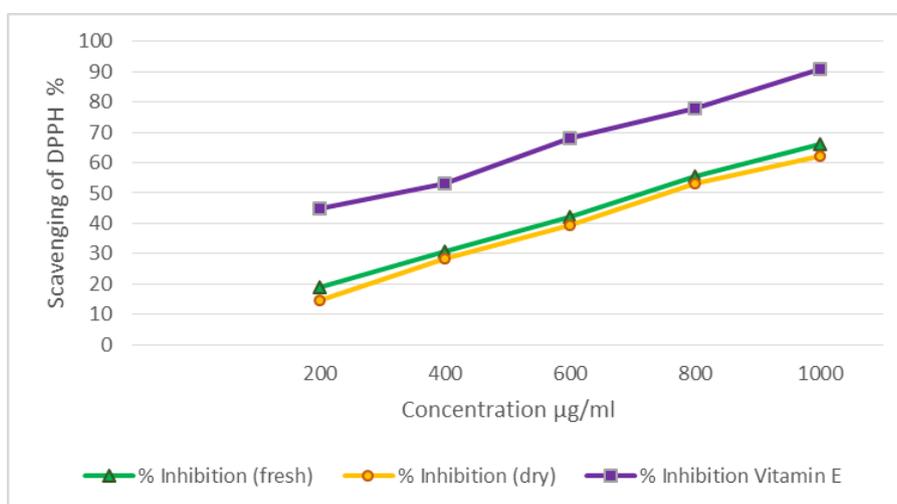


Fig. 7: DPPH radical scavenging of essential oils of fresh and dry *Zingiber officinale* compared to Vitamin E

3.2. Determination of IC_{50} :

IC_{50} value is inversely related to antioxidant capacity of the sample. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50%. Thus, the lower IC_{50} value the higher antioxidant activity (Sharififar *et al.*, 2007).

Results were obtained from a calibration curve of the extracts and vitamin E. The concentrations of EOs and vitamin E as a function of the percentages of DPPH inhibition were plotted in the Fig. 8 and Fig. 9 to graphically estimate the IC_{50} value. For comparative purpose, results showed a very potent antiradical activity of vitamin E with

an $\text{IC}_{50} = 309.5 \mu\text{g/ml}$. Nevertheless, essential oils of the *Zingiber officinale* provided lesser but satisfactory results (very highly significant difference $p < 0,001$). It is also noticed that the fresh ginger extract presents a higher antioxidant activity with an IC_{50} equal to 723 $\mu\text{g/ml}$, compared to the dry ginger extract with a value of 774 $\mu\text{g/ml}$, with $p < 0,05$.

Our results are almost similar to those of Stoilova *et al* (2007) who noted that the oleoresins, such as eugenol, shogaols, zingerone, gingerdiols, gingerols, diacetoxy-gingerdiol, found in ginger extract has a substantial scavenging capacity of 90% with an IC_{50} corresponding to 640 $\mu\text{g/ml}$.

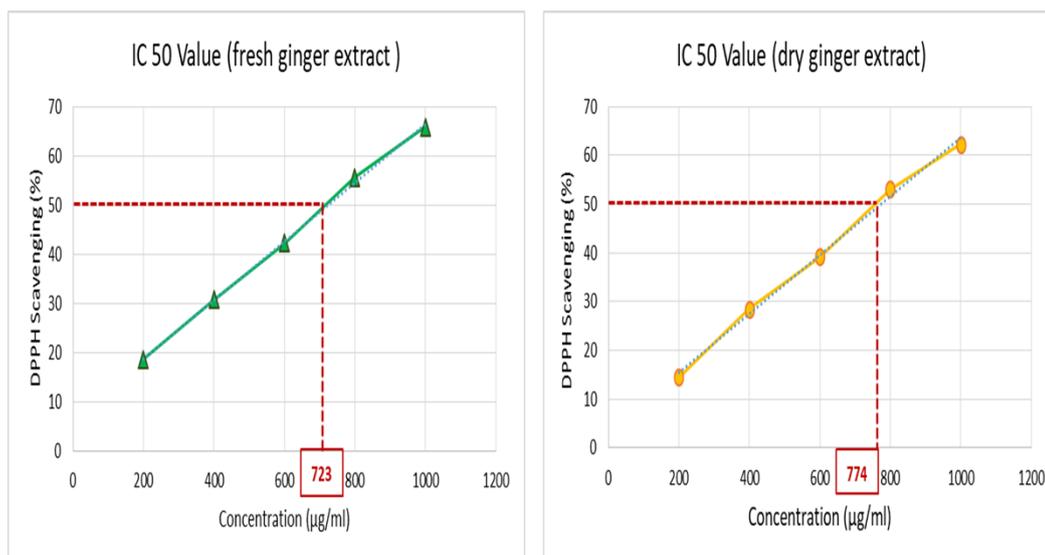


Fig. 8: IC₅₀ estimation of fresh and dry ginger extract.

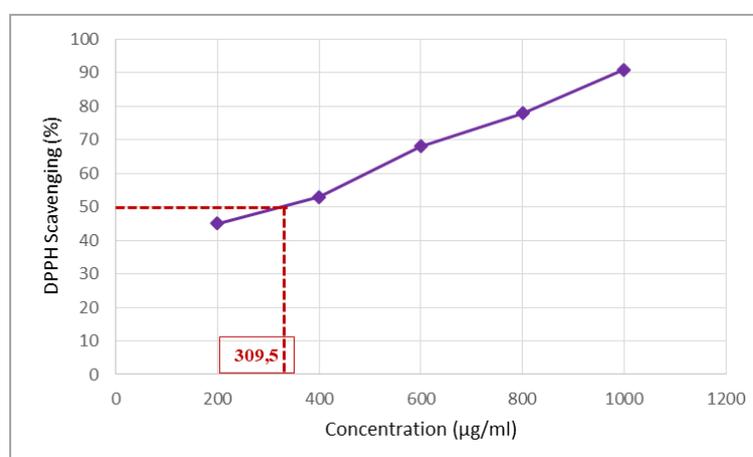


Fig. 9: IC₅₀ estimation of Vitamin E.

DISCUSSION

The EOs yields recovered after hydrodistillation show significant differences that could be explained according to (Kelen et Tepe, 2008) by the choice of the period of harvest in addition to the climate, the geographical zone, the genetics of the plant, the degree of freshness, the duration of drying and the method of extraction employed. These are the factors that can have a direct impact on the yield of EO.

EO samples have a specific density that is less than one (01), and are therefore classified as light oils. The refractive index varies essentially with the content of monoterpenes and oxygenated derivatives. High concentration of monoterpenes will give a high index according to some authors (Boukhatem *et al.*, 2016). Both acid number

values being lower than 2, is an indication of good conservation of the essence (low amount of free acids) (Kanko *et al.*, 2004). Drying decreases volatile compounds and pungent principles of ginger and thereby affects the taste and aroma (Amoah *et al.*, 2022 ; Jayasundara & Arampath, 2021). The variation of the antioxidant activity depends on several factors, such as; the level of phenolic compounds, electron donor functional groups, hydroxyl functions, as well as synergies between the active compounds that constitute each extract (Sharififar *et al.*, 2007)

Conclusion

Based on the in vitro results, the Ginger aqueous extract presented considerable antioxidant activity by

inhibiting DPPH. This ability may be due to tannins, phenolic compounds, alkaloids and saponin, glycosides, triterpenoids, flavonoids, oleoresins. The medicinal prospects of *Zingiber officinale* essential oil are therefore very promising. Further studies and clinical tests are needed to evaluate the in-vivo antioxidant potential of Ginger extract in various animal models. Also, this study could be complemented by other trials in order to develop appropriate approaches for a possible application on humans, in order to investigate whether it has other possible virtues (anti-cancer, antifungal, antibacterial, etc.) and to determine at what dose it can be safe for the consumer.

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ARABIC SUMMARY

الخصائص البيوكيميائية و النشاط المضاد للأكسدة لجذور الزنجبيل الجافة و الطازجة

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تشهد العديد من الموارد النباتية في الجزائر، بما في ذلك الزنجبيل (*Zingiber officinale*)، إحياء الاهتمام بالعبادات الغذائية للسكان المحليين من خلال ربطهم بمهاراتهم ومعارفهم التقليدية. الهدف من هذه الدراسة هو استخراج ودراسة بعض الخصائص الفيزيائية والكيميائية للزيوت الأساسية (زأ) لجذور الزنجبيل الطازج والجاف وتقييم نشاطهما المضاد للأكسدة في المختبر عن طريق تثبيط الجذور الحرة DPPH.

تم استخراج الزيوت الأساسية باعتماد طريقة التقطير بالبخار لعينات الزنجبيل الطازجة والجافة والذي نتج عنه عاندا قدره $0.42 \pm 0.017\%$ و $1.55 \pm 0.067\%$ على التوالي.

مكّن تقييم خصائصها الفيزيائية والكيميائية، بنفس الترتي ، وفقاً لمعايير AFNOR (معيان NF ISO 4731: 2006 NF T 75) - من العثور على النتائج التالية: معامل الانكسار: 1.47033 ± 0 ، 0011 و 1.4867 ± 0.0013 ؛ الكثافة: 0.8748 ± 0.00050 و 0.8838 ± 0.0005 ؛ الرقم الحمضي: 1.122 ± 0.08 و 1.629 ± 0.034 مجم KOH / جم ؛ مؤشر الإستر: 15.708 ± 0.39 و 18.411 ± 0.61 مجم KOH / جم.

أظهر تقييم القدرة المضاد للأكسدة عن طريق تثبيط الجذور الحرة DPPH أن الزيوت الأساسية للجذور الطازجة لـ *Zingiber officinale* تتميز بنشاط أعلى بشكل ملحوظ ($p > 0.01$) عن تلك المستخرجة من الزنجبيل المجفف. ولكن تبقى كلتا الزيتان أقل نشاطاً بصفة ملحوظة ($P > 0.001$) من فيتامين E والذي يبقى الأفضل مع $EC_{50} = 309.5$ مقارنة مع زأ للزنجبيل الطازج ($EC_{50} = 723$ ميكروغرام / مل) ؛ والزنجبيل الجاف ($EC_{50} = 774$ ميكروغرام / مل) ، أظهرت جميع النتائج الإحصائية للخصائص المدروسة فروقاً طفيفة بين نوعي الزنجبيل.

الكلمات المفتاحية: *Zingiber officinale*، الخصائص الفيزيائية والكيميائية، الزيت الأساسي، نشاط مضادات الأكسدة، التقطير المائي.