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Comparative study of the antioxidant activity of green teas marketed in Morocco and China

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ABSTRACT Tea is made from the processed leaves of Camellia sinensis, and is the most consumed beverage in the world and in Morocco after water. It is also one of the staple foods in Morocco and is consumed by all social classes. It is therefore essential to study its effects on health. This work contains a literature review that sums up the current body of knowledge concerning the composition of tea, its preventative and curative effects on different pathologies, and the potential risks associated to its consumption. The second part consists of a study of the antioxidant activity conducted on samples of commercially available green teas in Moroccan supermarkets and how they compare to green teas bought in China. The main observation of this study is that there is a large variability in the antioxidant activity of the commercialized teas. We hope that consumers, authorities and importers can make efforts to raise the quality of the imported and consumed teas so as to maximize the potential benefits of this beverage on the population.

KEYWORDS
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INTRODUCTION

Green tea, made from the leaves of Camellia sinensis, is a widely consumed commodity in Morocco, with 82,456 tons imported in 2019, and an annual consumption of nearly 2kg per capita. However, few studies have been carried out or published on the quality of tea available on the market and its comparability with teas consumed in other countries. This problem is all the more important when considering the active compounds in tea, which is believed to be one of the main sources of antioxidants for the Moroccan population, and its effects on consumers. The antioxidant activity of tea is often cited as a marker of quality and for commercial purposes because of its beneficial effects in the prevention of several pathologies related to oxidative stress. The purpose of this study is therefore to examine the antioxidant activity of several samples of teas marketed in supermarkets in Morocco and to compare them to samples marketed in China, which provides 98% of the tea sold in Morocco,

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MATERIALS AND METHODS

Samples

A total of six samples of green teas were studied, four of which were marketed in Morocco and two in China. The Moroccan samples come from 4 different brands (Car, Mkg, Slt and Vio) and two different types Gunpowder "" (Slt and Car) and Chun mee/Zhen mei "" (Vio and Mkg) purchased on January 18, 2020 in a department store in Rabat. Chinese samples of two different types Long Jing and Lao Shan Lu (Lj, Lsl) were purchased on July 18, 2018 at the ChongQing Tea Market.

Preparation of extracts

The extracts were prepared by infusing 20g of each sample in 300ml of tap water at 100°C for 15 minutes. The liquid was then filtered through a metal filter to retain the tea leaves and then through a paper filter using a Büchner funnel and a vacuum flask. The filtrate is then placed in a rotary evaporator (rotavapor) at 40°C to be concentrated. The concentrated extract is then freeze-dried, weighed and stored in sealed glass vials away from light. This operation is carried out in duplicate for each sample.

Free radical scavenging DPPH

DPPH or 2,2-diphenyl-1-picrylhydrazine is a stable free radical with a violet color that reduces to a yellow compound, the intensity of the color is inversely proportional to the reducing capacity of the antioxidant compounds present in the solution. Preliminary trials have allowed the preparation of concentration ranges from 0.1 to 3mg/ml. A volume of 50μl of each concentration was added to 2ml of a 0.0023% DPPH solution prepared in methanol. The mixture was incubated at room temperature in the dark for 20 minutes. Discoloration of this range and the negative control, containing only 50μl water and 2ml DPPH, is measured at 517 nm against a blank containing only methanol. Measurements are performed in triplicate for each of the two lyophilisates of each sample, for a total of 6 measurements per concentration of each sample. The inhibition percentage is calculated according to the formula :

$$Inhibition = \frac{DO_{\text{negatif control}} - DO_{sample}}{DO_{\text{negatif control}}} \times 100$$

The concentration responsible for the 50% inhibition of DPPH radicals (IC50) is determined using the concentration-dependent inhibition percentage curve.

Iron Reducing Power Test

The capacity of the extracts to transfer electrons from ferric iron (Fe3+) to ferrous iron (Fe2+) was determined according to the Oyaizu protocol [132]. A range of concentrations (from 0 to 3mg/ml) was prepared in water, 0.5ml of each sample, 2.5 ml of 1% potassium ferricyanide (K3Fe(CN)6) and 2.5 ml of phosphate buffer (0.2M, pH=6.6) were mixed and incubated in a water bath at 50°C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid was added to stop the reaction. The contents of each tube are then divided into 3 test tubes, 2.5ml per tube, to which 2.5ml of distilled water is added each. Finally, 0.5ml of iron chloride (FeCl3) is added immediately before the measurement of absorbance at 700nm. Optical density versus concentration curves are plotted and the IC50s are determined graphically.

RESULTS

The antioxidant activity of the samples was studied by evaluating their reducing power with respect to iron, and their antiradical activity with respect to DPPH. The mean concentrations leading to 50% inhibition (IC50) and their standard deviations (sd) for each sample are grouped in Table 1. These same averages are shown in Figures Figure Figure 1and Figure 2. Figure 3shows a correlation between the IC50 values calculated by FRAP and DPPH, with a Pearson correlation coefficient (r = 0.9427 and p = 0.0048).

All extracts were able to reduce the purple radical DPPH to yellow DPPH-H and were able to reduce Fe3+ to Fe2+.The extract of "Lsl" had the highest activity with (IC50 DPPH = 7.443 ± 0.142 μg/mL) and (IC50 FRAP = 36.723 ± 0.360 μg/mL), and "Car" had the lowest activity with (IC50 DPPH = 18.697 ± 0.789 μg/mL) and (IC50 FRAP = 77.167 ± 0.667 μg/mL) respectively.

As shown in Table 1and Figure 1and Figure 2, the order in which the samples were ranked according to their antioxidant potency was generally similar for both trials, in descending order :

- For DPPH: Lsl, Lj, Vio, Slt, Mkg, Car,
- For FRAP: Lsl, Lj, Slt, Vio, Mkg, Car

The ANOVA results for the FRAP measurements show that the differences between samples are significant (P < 0.0001) and Tukey's multiple comparison test shows significant differences

	DPPH	FRAP
Lsl	7,443 ± 0,142	36,723 ± 0,360
Lj	7,335 ± 0,376	39,550 ± 0,683
Vio	9,484 ± 0,082	49,971 ± 0,493
Tel:	11,193 ± 0,346	44,464 ± 0,513
Mkg	13,772 ± 0,992	51,620 ± 0,391
Because	18,697 ± 0,789	77,167 ± 0,667

Table 1 IC50 (μg/ml) of the lyophilisates of the different samples.

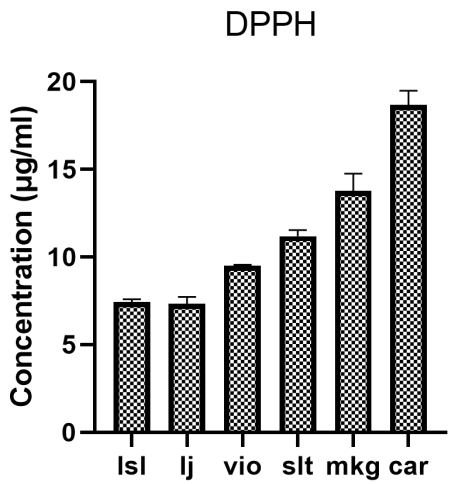

Figure 1 IC50 (μg/ml) of samples versus DPPH

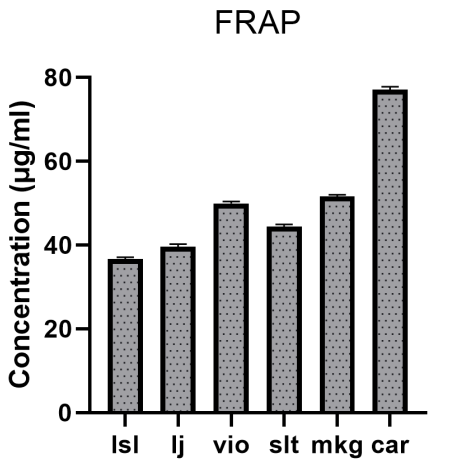

Figure 2 IC50 (μg/ml) of the samples with respect to iron reduction capacity

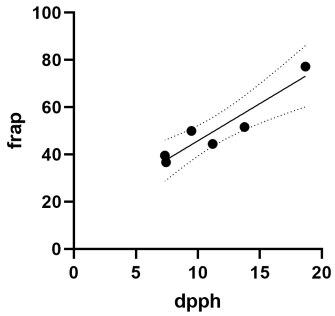

Figure 3 Correlation of DPPH and FRAP values

between all samples. For DPPH the ANOVA also shows a significant difference between the means ($p < 0.0001$), Tukey's multiple comparison test shows significant differences between each pair of groups except between Lsl and Lj ($p>0.99$). The Chinese samples, Lsl and Lj, show significantly lower IC50s than the Moroccan samples, between 11.05% and 52.41% for FRAP and between 21.52% and 60.71% for DPPH. Pearson's coefficient shows a strong correlation between the price of tea and its IC50 with ($r = -0.694$, $p = 0.125$)

DISCUSSION

The antioxidant activity of tea has long been studied and is widely recognized. The compounds responsible for this activity "antioxidants" were originally studied simply for their ability to preserve food longer. Their definitions have gradually changed over time from "Any substance that, when present in low concentrations relative to an oxidizable substrate, is capable of significantly slowing or inhibiting the oxidation of that substrate" by Halliwell and Gutteridge in 1995 Halliwell (1995)[133]; through several gradual changes to the definition by Apak et al. in 2016 Apak *et al.* (2016)[134] "natural or synthetic substances that could prevent or retard cellular oxidative damage caused by physiological oxidants with distinctly positive reducing potential, including reactive oxygen species (ROS) reactive nitrogen species (RNS) and free radicals". These definitions demonstrate the role of antioxidants at the cellular level and its relationship to oxidative stress and free radicals, and their potential effects on human health. In the case of green tea, it is mainly attributable to polyphenols and more specifically to gallocatechinsZhu *et al.* (2001); Wan *et al.* (2008); Yang *et al.* (2002); Nanjo *et al.* (1996)[51]-[54]. However, it is impractical to determine the amount of each of several hundred active compounds present in different foods, so methods to measure "total" antioxidant activity are used to quantify itBenzie and Devaki (2018) [135]. Measuring antioxidant activity is therefore not only important to determine the effectiveness of functional foods in the treatment of diseases related to oxidative stress, but also to evaluate and compare their quality.

This activity was tested by two complementary methods in our study, the DPPH trapping test and the iron reducing power test. This choice of method is based on their different reaction mechanisms; the FRAP assay is based solely on an electron transfer reaction, whereas the DPPH assay is based on both electron transfer and hydrogen atom transfer (Hydrogen Atom Transfer/Single Electron Transfer, HAT/SET) Sun *et al.* (2018)[136] The combination of the two methods allows us to determine that our samples use a mixed mechanism for their antioxidant activity. The strong correlation between DPPH and FRAP values ($r = 0.9427$) shows that both techniques are valid for measuring antioxidant activity.

The significant difference between the test results of the different samples (calculated by ANOVA with $p<0.0001$) shows that the sensitivity of these tests is sufficient to differentiate and compare the samples. The two samples purchased in China show very similar IC50s for FRAP and a statistically insignificant difference for DPPH, and are also the two samples with the most potent antioxidant activity (IC50s between 11% and 60% lower than the samples from Morocco).

These results are in agreement with the literature, several Chinese studies Zhao *et al.* (2019); Zhang *et al.* (2013); Yashin *et al.* (2011)[137]-[139] have found that Long Jing tea has an important antioxidant activity and higher than the average of green teas which places it in first position among the teas compared in two studies Zhang *et al.* (2013)[138], [139], and in third position among 30 teas in another studyZhao *et al.* (2019) [137]. Similar results are found for Laoshan-lu tea in other studies Qian *et al.* (2010); Wang *et al.* (2009)[140], [141]. Unfortunately we could not find any publications concerning the antioxidant activity of teas imported into Morocco, Gunpowder and chun mee, this is probably due to the fact that these two types of tea are almost exclusively consumed in Maghreb countries which makes their inclusion in teas studied in other countries unlikely.

Samples purchased in supermarkets in Morocco show a wide variation in antioxidant activity, from 9,484 μg/mL to 18,697 μg/mL for DPPH and from 49,97 μg/mL to 77,16 μg/mL. The origin of this variation is not clear with the data available, but several hypotheses can be made to explain, at least in part, these results. The correlation between activity and tea price indicates that the raw material used to make the cheapest teas would be less rich in antioxidants, this could be due to the fact that the tender leaves and buds that have a higher price also contain more polyphenols.

Another contributing factor could be the oxidation of the tea after the end of production, this hypothesis is supported by the observation of the color of infusions and extractions which appears more brownish in cheaper teas and greenish in more expensive teas (Figure 4and Figure 6Concentrated extracts of Vio and Car), this color is an indicator of oxidation that can come from several sources:

- The integrity of the leaves after processing, more expensive teas use gentler rolling methods to keep the leaf intact, reducing the surface area in contact with air and slowing down oxidation.
- Unsuitable storage conditions to reduce costs, in the open air or at high temperatures.
- We also note an absence of the date of harvest on the majority of the packages, replaced by a packaging date, which makes it impossible to determine the storage life.

The differences between the activities of teas purchased in China and Morocco, in addition to the above reasons could come back to the different organoleptic characteristics sought by consumers in the two countries, the Chinese who generally drink tea without sugar are looking for a tea with sweet and Umami tastes with a minimum of bitterness and astringency, which encourages the production of a more "green" and less oxidized tea. Moroccans, on the other hand, consume it decocted with sugar and other herbs, and therefore seek a very astringent and bitter tea whose taste will not be masked by sugar. Importers also seek a tea that keeps more easily and longer, which pushes producers to use higher temperatures during production which could lead to deterioration of the active compounds in the tea.

However, the 11% difference in activity between teas sold in China and tea sold in Morocco with the most potency, as well as

the wide variation in activity of teas sold in Morocco, indicates the possibility that teas with comparable activity to those sold in China are also sold in Morocco but are not among the teas tested in this study.

The results of this study thus confirm the antioxidant activity of green tea marketed in Morocco, although the degree of this activity varies greatly depending on the quality of the tea. Special attention should therefore be paid to the quality of imported tea to maximize the effect of this commodity on Moroccan consumers. In the same vein, greater transparency with respect to production, not packaging, dates, which can be months or even years apart, will allow consumers to make better informed choices.

LIMITATIONS OF THE STUDY AND OUTLOOK

The study has several limitations that need to be addressed in the future. First, although the number of samples studied may give some idea of the magnitude of variation in antioxidant activity in teas marketed in Morocco, it would be interesting to use a larger number of samples to form a more complete picture of the market. Also, the broadening of the study's spectrum to include other tea activities as well as the determination of contaminants (heavy metals, mycotoxins, pesticides, ...) is important to assess the real impact of its consumption on the population. Other important aspects are related to Moroccan tea preparation traditions and the way tea is prepared, such as the effect of brewing by decoction compared to infusion, the effect of adding sugar and the timing of its addition (before or after decoction) on the composition and activity of the tea. The effect of the addition of other herbs to the drink such as mint or wormwood and their effects on toxicity and the presence of potential synergistic effects.

CONCLUSION

Tea is rich in minerals and active compounds, especially polyphenols which are powerful antioxidants. Numerous studies have proven their effects on humans for the prevention of cancers. These same polyphenols also have other interesting activities, such as an antihypertensive, lipid-lowering, neuro-protective, anti-microbial, photo-protective, and anti-diabetic effect. It also contains other psychoactive compounds such as caffeine and L-theanine which make it one of the only drinks known to have both an exciting and calming effect, allowing it to improve mental performance without the side effects of caffeine. In addition, tea appears to have no major adverse effects or drug interactions when consumed in moderation by healthy individuals and as long as it undergoes strict quality controls for the presence of contaminants and above-standard levels of pesticides. All these reasons, in addition to the fact that it is already widely appreciated and consumed by the population, should push us to try to maximize its positive effects. To do this it is important to first have an idea about the quality of tea marketed in Morocco and compare it to those sold abroad to validate the applicability of the results obtained by researchers from other countries to tea marketed in Morocco.

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