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Comparative Study of the Influence of the Micellar and Ethanol Medium on the Antiradical Activity of the Aqueous Extracts of three Chewing Stick used in Benin for Oral Hygiene

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Abstract Free radicals are risk factors that can lead to certain oral infections. The contributions of antioxidants is necessary to fight effectively against these free radicals, in the oral cavity. Plants being recognized as a source of natural antioxidants, chewing stick still used today in several developing countries can serve as a remedy to fight against these free radicals. Thus, in this study, we studied the antioxidant activity of plants used as a chewing stick in Benin by the DPPH method, both in ethanolic and micellar medium. The results show that the DPPH method in micellar medium is a credible alternative to the conventional method which takes place in ethanolic or methanolic medium. Depending on the concentration of antioxidant present, the percentage inhibition of DPPH increases with the concentration of CTAB to a given value where it begins to fall. The activity also depends on the number of hydroxyl groups provided by the phenolic compounds.

Keywords Antioxidant; Micelle; chewing stick; phenolic compounds

Introduction

Oxidative stress has been described as a process derived from the inability of endogenous antioxidants to trap free radicals and has been associated with many pathologies such as aging, cardiovascular diseases, neurodegenerative diseases, cancer, regional pain syndrome complexe. This evidence suggests that free radicals play a key role in the development of several pathological conditions [1]. Free radicals and reactive oxygen species (ROS) are responsible for the inflammatory response. Periodontal pathogens may induce overproduction of ERO and thus cause collagen and periodontal tissue degradation. Recently, work has shown that imbalances in levels of free radicals, reactive oxygen species and antioxidants in saliva may play an important role in the development of dental caries [2].

Periodontal tissue depends on natural antioxidants to overcome this oxidative stress and maintain homeostasis. These observations suggest that antioxidant-rich diets may inhibit the development and progression of periodontal disease, particularly in individuals exposed to environmental and dietary sources of oxidative stress [3, 4]. Several studies also report that the decrease in antioxidant activity of cerebral fluid and saliva is associated with the development of periodontitis [3, 5]. Polyphenols can help increase the antioxidant activity of oral fluids. Indeed, the

administration of tea polyphenols, holding green or black tea in the mouth for 2 to 5 min increases the antioxidant capacity of saliva [6]. For example, the use of some popular chewing stick, most of which contain different substances, can keep the oral cavity generally healthy [7]. Even though many people have abandoned the traditional use of chewing stick and have adapted to the conventional method of brushing teeth, some others still make use of them as a daily ritual to maintain oral hygiene. This is particularly true in developing countries where the economy, customs, religion and the availability of oral hygiene tools play a role in their continued use. Vegetable tooth brushes have long been used in the Greek, Roman, Jewish and Islamic empires [8].

Studies on the antioxidant activity of these different plants used as chewing stick are very important nowadays because of the role that the use of these plants can play in the preservation of oral health. Several methods have been developed to evaluate the antioxidant potential of plant extracts. They are based on the determination of products resulting from oxidation or, on the contrary, measure the effectiveness of a substance to trap free radicals, often by giving a $H^{\bullet}$ form [9]. Among them, the assay using the free radical, 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) is the most widely used in vitro test [10; 11]. This radical reacts with both electrons and hydrogen donors [12], in other words, the DPPH test is based on both electron transfer (ET) and hydrogen transfer (HAT) [13]. These tests are often carried out in organic solutions or mixed solvents due to the insolubility of the species used in water [10]. Very few systematic studies have been reported in the literature on the use of the pure aqueous medium in the study of the $DPPH^{\bullet}$ scavenging ability of antioxidants. So, it is necessary to develop new techniques to overcome this limitation. To this end, the use of micelle systems is a simple alternative because they are known to improve the solubilization, dissolution and bioavailability of pharmacologically bioactive molecules in aqueous solutions. [14]. Other researchers have also proposed kinetic parameters that can provide more complete information on the behavior of an antioxidant and suggested that kinetics might be more important than total antioxidant capacities determined at a fixed point [15]. The present work investigates these new methods in order to better evaluate the antioxidant activity of some plants used as a plant brush in Benin, while using gallic acid as a study model. Polyphenols are known for their antiradical activity and for that we first made the phytochemical screening stick. Then the second part is dedicated to study of the influence of the medium (CTAB and ethanol) on the percentage and the rate of inhibition of $DPPH^{\bullet}$ by gallic acid. From the result, the best concentrations of CTAB were choosing to study the antiradical activity of the chewing stick and the results are discussed in the third part.

Material and Method

Chemical

Formalin, chlorhydric acid, sodium sulfate iron chloride III, ether, ammoniac, hydrochloric alcohol, magnesium, isoamyl alcohol and sulfuric acid were used for phytochemical screening. 2,2-Diphényl-1-picrylhydrazyle ($DPPH^{\cdot}$), Bromure de Céthyltriméthylammonium (CTAB), acetate buffer (0,1 M ; pH=4,6), gallic acid were used to study the antiradical activity.

Plant material

The plants under study are *Anogeissus leiocarpa* (root), *Burkea africana* (stem) and *Murraya paniculata* (stem) collected in the central region of Benin republic

Method

Extraction of plants

The plants, once harvested, were dried in the laboratory. They were then crushed to make two types of extracts, namely macerated and aqueous decoction. The aqueous macerate was obtained by maceration for three successive days, taking care to renew the solvent each day while the decoction was prepared by heating to boiling. The filtrates obtained were concentrated on a rotary evaporator and then in an oven at 40 ° C. for 3 days.

Phytochemical Screening

The determination of the presence of secondary metabolites has been carried out by methods used by authors [16, 17, 18]. Screening was performed on both whole plants and each extract.

Gallic tannin: Stiasny's reaction

To 30mL of infused (5%), we added 15mL of Stiasny's reagent (10mL of 40% formalin + 5mL of concentrated 37% HCl). The whole is heated in a water bath at 90 ° for 15 min. The appearance of precipitate indicates the presence of catechin tannins. For gallic tannins, the previous solution was filter and the filtrate was Saturated with sodium acetate or powdered sodium sulfate (desiccant). Then we added drop wise 1mL of 1% iron chloride III. The development of a blue-black hue indicates the presence of gallic tannins, not precipitated by the Stiasny reagent.

Coumarins

The solution to be analyzed is obtained after maceration for 24 hours (store the solution in a cold room) with 1 g of vegetable powder in 20 ml of ether. Filter and make up to 20 mL. Evaporate to dryness 5mL of the filtrate. Add 2mL of hot water to the residue. Share the solution in 2 test tubes. In one of the tubes add 0.5 mL of 25% of ammoniac. Mix and observe fluorescence under UV at 366 nm. The presence of coumarins is indicated by intense fluorescence in the tube.

Flavonoids

Flavonoids were identified by the cyanidin reaction which consisted in introducing into a test tube containing 5 ml of 5% infused 5 ml of hydrochloric alcohol, a few chips of magnesium and 1 ml of isoamyl alcohol. The appearance of a pink-orange (flavones) or purplish pink (flavanones) or red (flavonols, flavanonols) colony collected in the supernatant layer of isoamyl alcohol indicates the presence of flavonoids.

Anthocyanins

At 5% infusion, 5mL of 10% sulfuric acid and 5mL of 50% ammoniac are added. The appearance of a coloration after acidification which turns to purplish blue in basic medium indicates the presence of anthocyanins.

Study of the antiradical activity in micellar and in ethanolic medium:

The antioxidant power was measured in two different media: a micellar medium (Cethyltrimethylammonium: CTAB) and an ethanolic medium according to the method proposed by [19] with some modifications.

Different concentrations of the CTAB emulsifier were prepared by dissolving the emulsifiers respectively in 0.05M phosphate buffer solutions at pH = 7.2 and 0.1M acetate at pH = 4.6. The DPPH solutions was prepared in ethanol and those of the plant extracts and the reference compound (gallic acid) were prepared in the emulsifier solutions.

In general, 1.6 mL of the emulsifier solution (CTAB) and 0.2 mL of the DPPH stock solution prepared in ethanol were mixed in a cuvette. The mixture is stirred for a few seconds and then introduced into the spectrophotometer. After reading the first value of absorbance at 528 nm, the cuvette was removed to add 0.2 mL of the antioxidant solution prepared in the emulsifier solution and then returned within one minute ($0 < t < 15$ s) on the spectrophotometer to study the kinetics of the reaction. In the mixture, the initial concentration of emulsifier is 0; 2; 5; 10; 20 and 60 mM while the initial concentration of DPPH is 50 µM and that of the antioxidant is 0; 10; 25; 50 and 100 µM.

The DPPH in the micelle aqueous solution without antioxidant was considered as blank and the study in the ethanolic medium was carried out under the same conditions by taking 1.6 mL of ethanol instead of the CTAB except that the absorbance is read this time at 516 nm, DPPH absorption domain in ethanol.

Experiments were done in triplicate (three different cuvettes of each set of concentrations) and the results were averaged.

Kinetic study:

The reaction of $DPPH^{\cdot}$ with antioxidants occurs through two mechanisms namely, a hydrogen atom transfer mechanism (HAT) and an electron transfer mechanism (ET), from the phenolic compound to the $DPPH^{\cdot}$. In methanol and in ethanol, it is the electron transfer mechanism that predominates. Equations (1), (2) and (3) express the different reactions that may occur:

$$[DPPH^{\cdot}] + [AH] \leftrightarrow [DPPH - H] + [A^{\cdot}] \quad (1)$$

$$[DPPH^{\cdot}] + [A^{\cdot}] \leftrightarrow [DPPH - A] \quad (2)$$

$$[A^{\cdot}] + [A^{\cdot}] \leftrightarrow [A - A] \quad (3)$$

The visible absorbance of the $DPPH^{\cdot}$ decreases rapidly in the very first minutes, resulting from the mechanism of transfer of labile hydrogen atoms of the antioxidant: it is fast step. This step is followed by a second stage of slow disappearance of the $DPPH^{\cdot}$. Only the fast step is subjected to the kinetic study.

Thus, the reaction rates were determined by the same procedure as above, except that the absorbances are read every 15 seconds for 2 minutes. In view of the kinetics of the reactions, only the initial reaction rate after the first 15 seconds were evaluated by the following formula (4):

$$v = -\frac{[DPPH^{\cdot}]_{i+1} - [DPPH^{\cdot}]_i}{t_{i+1} - t_i} \quad (4)$$

$[DPPH^{\cdot}]_{i+1}$ and $[DPPH^{\cdot}]_i$ are the concentrations of $DPPH^{\cdot}$ at times t_{i+1} and t_i.

Experiments were done in triplicate (three different cuvettes of each set of concentrations) and the results were averaged.

Results and Discussions

Identification of phenolic compounds

The results of the phytochemical screening of whole plants as well as those of macerated and decocted water are recorded in Table 1. From the analysis of this table, it appears that all these plants have a diversified range of phenolic compounds. In particular, the various extracts of *B. africana* and *A. leiocarpa* have proved qualitatively the richest in these compounds. This study also showed that the composition of secondary metabolites may vary depending on whether the whole plant or one of these extracts is used. Coumarins are only found in *M. paniculata*. Indeed coumarins including aesculin are used in oral care for the treatment of periodontitis and prevention of bleeding [20].

Flavonoids have been detected in all plants, which in principle could give them good antimicrobial activity. Indeed, flavonoids have vitamin P properties at the level of the capillaries that they reinforce and allow to reduce the frequency of minor hemorrhages.

According to [7, 21], among the criteria for choosing a plant as a plant brush are the taste and texture of the plant. The presence at the level of the studied plants of the tannins, recognized for their astringency, could explain the reason for their traditional choice as chewing stick. Their presence in these plants is all the more important as they act preventively against microbial fermentations, by precipitating the proteins from the bacterial plaque and are therefore used in mouthwashes and toothpastes.

Table 1: Result of phytochemical screening

Secondary metabolites	**Plants**								
	M. paniculata			***A. leiocarpa***			***B. africana***		
	1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Tannin	-	+	+	+	+	+	+	+	+
Anthocyanins	-	-	-	+	+	+	-	-	+
Coumarins	+	+	+	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+

+: Present; -: Absent; 1: Whole plant; 2: aqueous macerate; 3: aqueous decoction;

Comparative study of the antiradical activity of gallic acid in micellar medium and in ethanolic medium:

It is known that a micelle medium can play a major role on the chemical behavior of the antioxidant agents [14, 22]. The natural polyphenols like flavonoids can usually exert their antioxidant action by three mechanisms including hydrogen atom transfer, single electron transfer to free radicals, and finally metal chelation. These mechanisms are affected by antioxidant structural features, solubility, partition coefficient and solvent composition [14, 23]. Furthermore, the behavior of many antioxidants in micro-heterogeneous micellar environments, which are more relevant to biological system, has not been well elucidated yet [24, 25].

Influence of CTAB concentration on $DPPH^{\cdot}$ absorbance

Figure 1 shows the behavior of $DPPH^{\cdot}$ in the different micellar media, in the buffer as well as in ethanol. This study allowed us to note the instability of $DPPH^{\cdot}$ in the micellar media and in the buffer, which implies a reactivity of the

DPPH· vis-à-vis these environments. In the acetate buffer used to prepare the CTAB, taken as a reference for a good comparison, the absorbance varied by about 6.95%. Thus, for a concentration of 2 mM, the curve reflecting the variation of the absorbance is below that of the buffer. Indeed, after 15 min, the absorbance varies from 9.7% in the micellar medium to 2 mM CTAB compared to the initial absorbance. For the other CTAB concentrations (5 mM, 10 mM, 20 mM, 40 mM and 60 mM), the absorbance variation curve is above that of the buffer.

For all these CTAB concentrations, the variation in absorbance is between 3.07% and 4.34%. It can therefore be concluded that at the edge of the critical micellar concentration of CTAB which is 2 mM according to [10], *DPPH* · is unstable while above this critical micellar concentration, it is much more stable. It should also be noted that the micellar medium absorbance is higher than that in the buffer, which could mean that the micellar medium allows a better dissolution of the *DPPH·*. These observations led us, for the rest of this study, to wait about 2 minutes for the stability of the CTAB-*DPPH·* mixture before the addition of the antioxidant, otherwise, the true antiradical power of the antioxidants studied would be poorly evaluated.

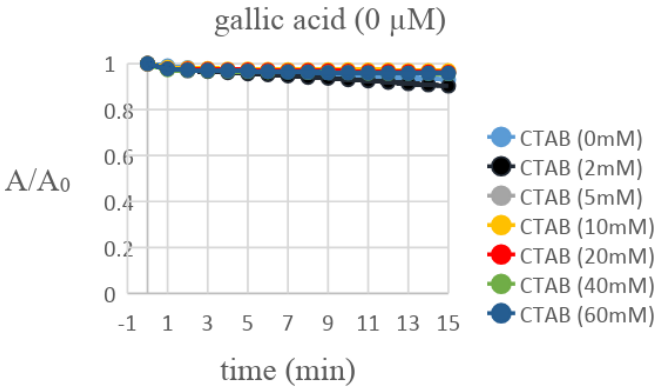


Figure 1: Variation in absorbance of DPPH· as a function of CTAB concentration

Were A_0 and A are the absorbance of the blank and the absorbance in the presence of the sample respectively

Influence of the reaction medium on the antiradical activity of gallic acid:

Figure 2 shows the DPPH ° inhibition curves for different concentrations of gallic acid (10 to 100 µM) in different media where the concentration of CTAB varies from 0 to 60 mM and then in a purely ethanolic medium.

For a concentration of 10 µM gallic acid, it was found, after 15 minutes that the percentage inhibition increases from 22.84 to 34.17% when the concentration of CTAB in the medium increases from 0 to 2 mM. But when the concentration of CTAB is greater than 2 mM (critical micelle concentration), that is to say 5 to 60 mM, the percentage inhibition decreases progressively from 32.90 to 27.2%. Meanwhile, in the ethanolic medium, the percentage inhibition is the lowest with a value of 21.28%.

For a gallic acid concentration of 25 µM, the percentage inhibition increases from 36.82 to 55.76% for CTAB concentrations ranging from 0 to 40 µM while for a concentration of 60 mM, the percentage of inhibition decreases to 41.09%. In the ethanolic medium, the inhibition remains always weak compared to the micellar media and is similar to that in the buffer medium.

As for the concentration of gallic acid of 50 µM, it induced an inhibition ranging from 37.38 to 64.73% for an increasing concentration of 0 to 40 mM CTAB and drops to 61.24% when the concentration of CTAB to 60 mM. In the ethanolic medium, this same concentration of gallic acid caused an inhibition of 52.78%. This inhibition, although greater than that obtained in the buffer, however, remains lower than those obtained in the CTAB.

The highest percent inhibition was obtained with a concentration of 100 µM gallic acid. This percentage of inhibition is 40.93% in the buffer medium and increases from 81.16 to 90.60% when the CTAB concentration increases from 2 mM to 60 mM. The inhibition in the ethanolic medium is as high as those in the CTAB and is of the order of 87.34%.

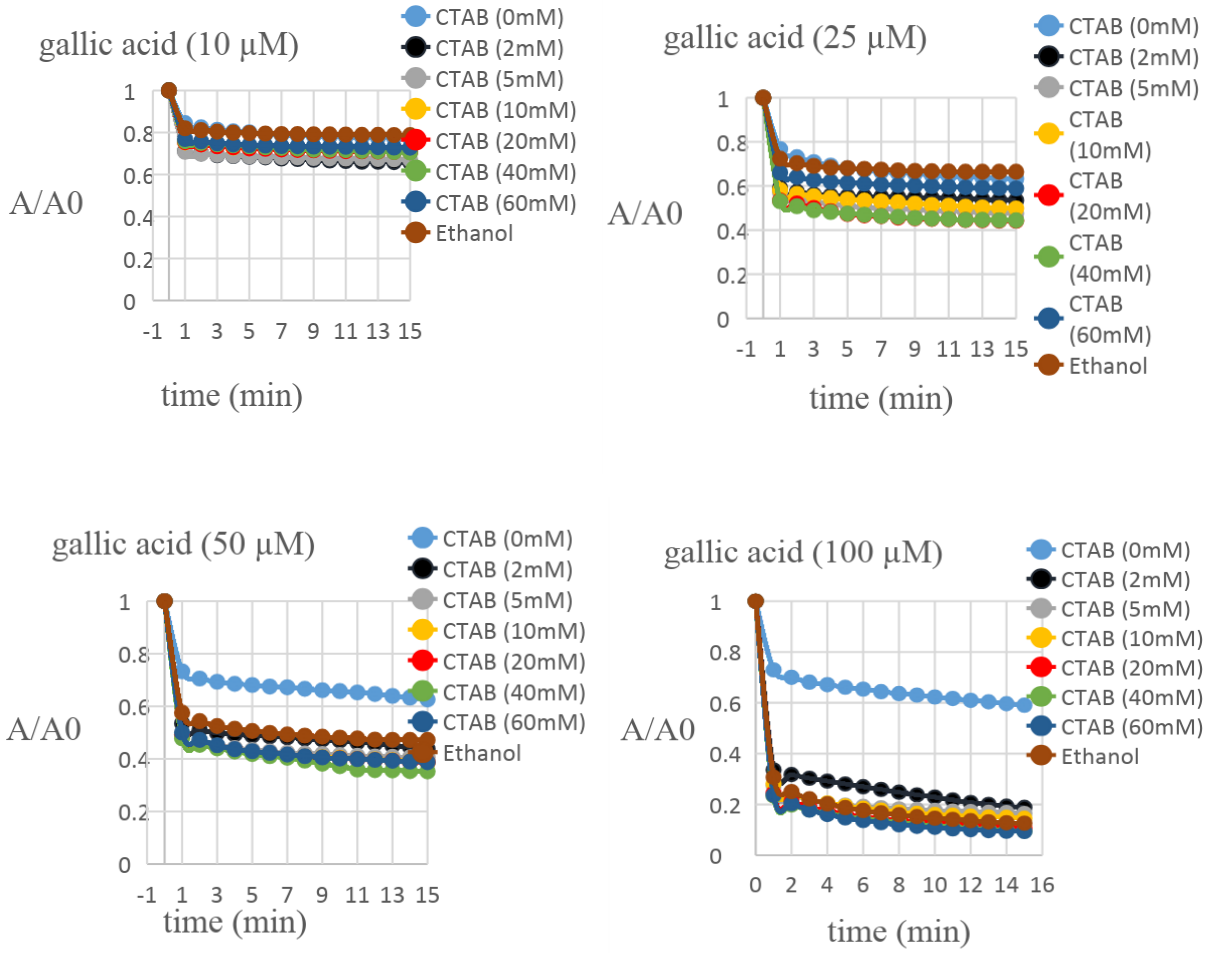


Figure 2: Curves of variation of DPPH inhibition by gallic acid at different concentrations of CTAB

In general, it should be remembered that the reaction of gallic acid, object of the present study, has an antiradical activity in the CTAB, slightly above that in ethanol. This shows that the reaction of DPPH with antioxidants can be carried out in aqueous media with excellent results. The percentage of inhibition depends on the concentration of CTAB in the medium.

Influence of CTAB concentration on antiradical activity:

Figure 3 shows the variation curves for DPPH inhibition depending on the concentration of CTAB for each concentration of gallic acid.

Indeed, for a given concentration of antioxidant, the percentage of inhibition increases with the concentration of the CTAB to a given value where it begins to decrease. The authors [19], made similar remarks when studying the influence of SDS on catechin antiradical activity. This observation could be justified by the fact that CTAB is a salt that dissociates completely in water, but at low modalities. Indeed, at a low concentration, the micelles are more or less spherical (globular) and at higher concentrations there are cylindrical micelles, lamellar, vesicles ... etc., the micelles are more compact [26].

It should also be noted that CTAB concentrations of 2 mM and 5 mM, 10 mM and 20 mM then 40 mM and 60 mM had almost the same effect. This led us, for the rest, to continue the study with the concentrations 2 mM, 20 mM and 40 mM CTAB.

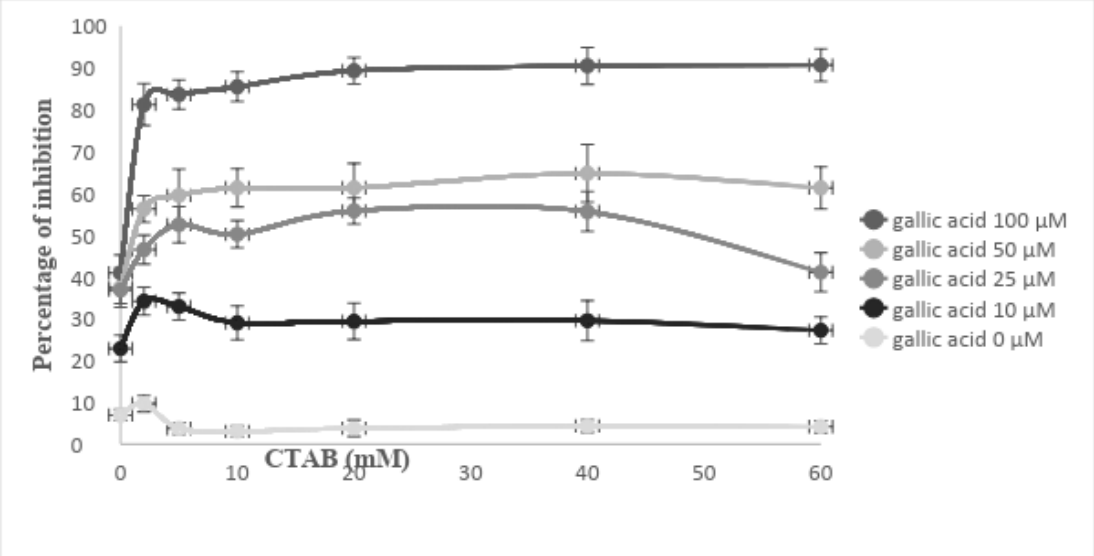


Figure 3: Curves of variation of percentage inhibition as a function of the concentration of CTAB

Influence of CTAB concentration on antiradical activity

The antiradical power of gallic acid was appreciated by the determination of gallic acid concentration that inhibit 50% of $DPPH^{\cdot}$ noted IC_{50} in both micellar and ethanolic medium. The IC_{50} is determined graphically by linear regression of the graphs of the percentages of inhibition of free radicals as a function of the concentrations of the extracts (figure 5). The percentage of inhibition was calculated by the formula (2)

$$P = \frac{Ab-Ae}{Ab} X100 \qquad (2)$$

With P: percentage of trapping; Ab: absorbance of the white; Ae: Absorbance of the sample

The results, expressed as µM are shown in figure 4.

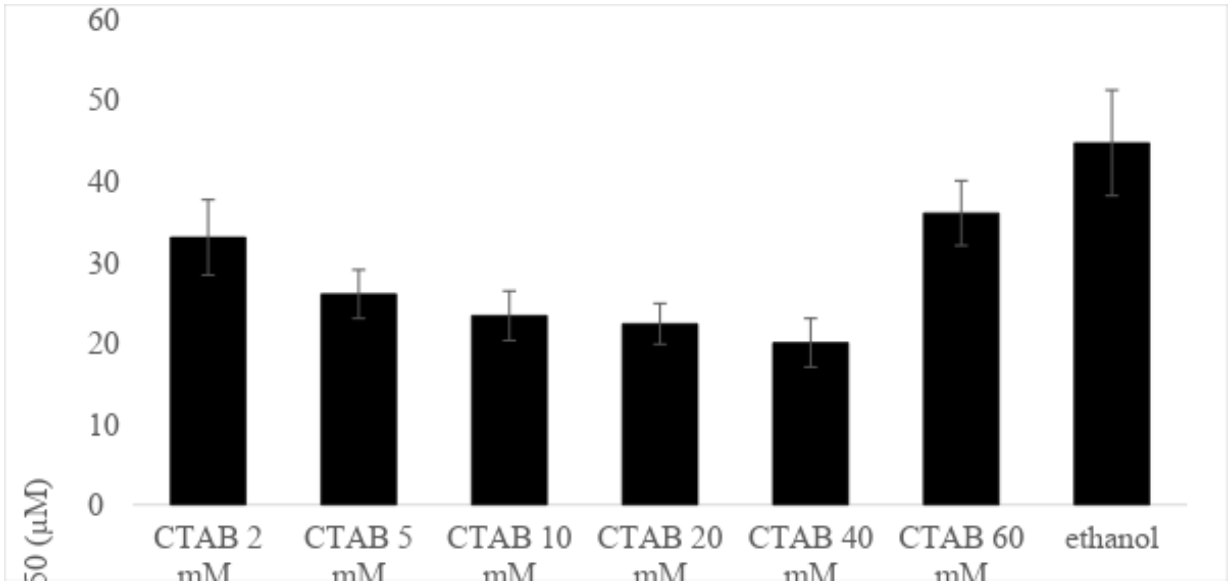


Figure 4: IC_{50} of gallic acid in micellar and ethanolic medium

In the absence of the CTAB, the inhibition percentage does not reach 50% whereas in the presence of the latter, the inhibition rate goes up to 80 or even 90% as indicated in figure 5. Increasing the concentration of CTAB from 2 to 40 mM, the IC_{50} decreases from 33,000 ± 2,645 µM to 20,000 ± 2,000µM, which means that the antiradical activity increases. On the other hand, for a concentration of 60 mM, there is an increase of the IC_{50} which is 36,000 ± 2,000

µM. In a purely ethanolic medium, a higher IC_{50} (44,667 ± 2,516 µM) is noted than in the presence of CTAB, although the final inhibition percentage is of the order of 90%. This observation is due to the fact that the CTAB allows an acceleration of the reaction. Once again there is a confirmation that the antiradical activity grows up with the increase of CTAB concentration to a certain level

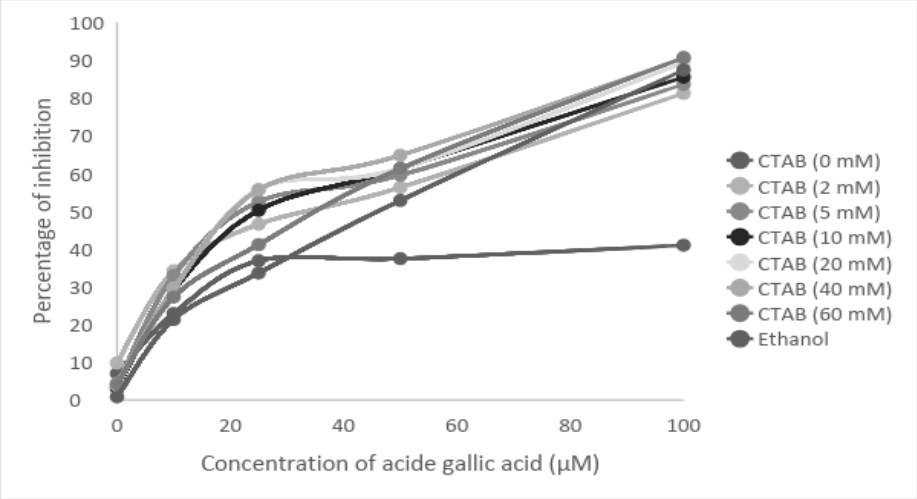


Figure 5: Variation of percentage of inhibition as a function of the concentration of gallic acid

Kinetic aspects of the DPPH reduction by gallic acid

Reaction time is an important factor that influences the inhibitory capacity of DPPH by antioxidants [27, 10]. Indeed, free radicals are very reactive chemical species and characterized by a short life span [1]. The reaction of gallic acid with the free radicals of DPPH being a quick reaction, we evaluated the reaction rate after the first 15 seconds. The results expressed in µM/S are shown in figure 6.

As before, for each concentration of gallic acid, the rate of the reaction increases with increasing concentration of CTAB to a level where it begins to decrease. This could be explain by the fact that inter and intramicellar diffusions are the rate-limiting steps for the antioxidation reaction conducted in micelle systems [28].The initial speed thus calculated in micellar medium is far better than that in ethanolic medium, although after 15 minutes of reaction, the percentages of inhibition in the two media is close.

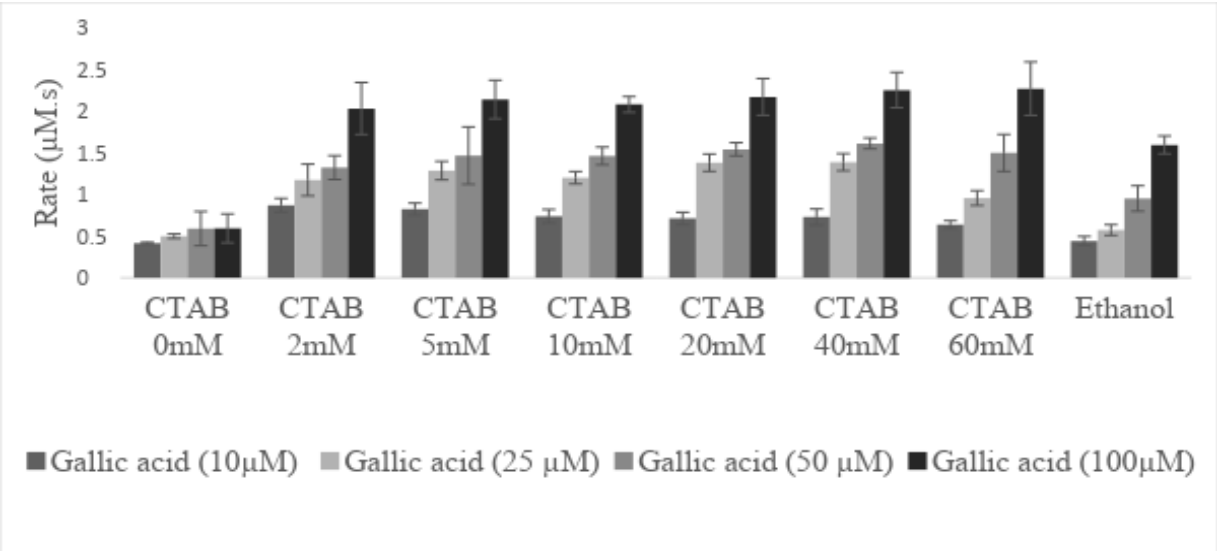
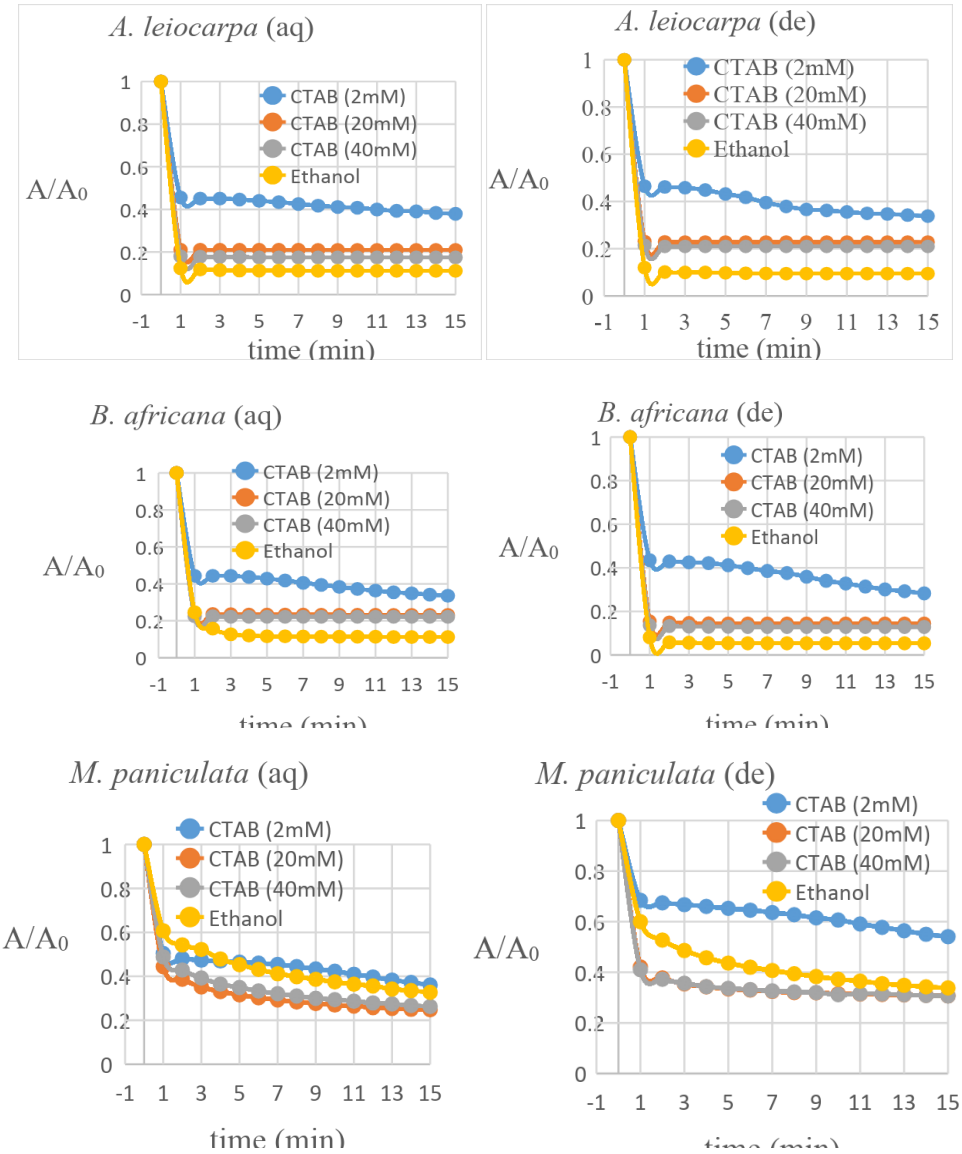


Figure 6: Rate of disappearance of DPPH as a function of the concentration of CTAB at different concentrations of gallic acid

Influence of the reaction medium on the antiradical activity of the plant extracts

This study was carried out in micellar medium with 2 mM, 20 mM and 40 mM CTAB then in ethanolic medium and the extracts were prepared at 1 mg/mL in view of the results previously obtained. The concentration of DPPH is maintained at 50 µM to allow comparisons with gallic acid taken as study model.

The curves showing the disappearance of DPPH in the presence of macerate and aqueous decoction of *A. leiocarpa* in media at different concentrations of CTAB as well as in ethanol are contained in Figure 7. Unlike the case of gallic acid, the inhibition in ethanol is significantly better than that in the CTAB for the three extracts studied. As before, the percent inhibition increases as the concentration of CTAB increases. Thus, when the concentration of CTAB increases from 2 to 40 mM, the percentage of inhibition increases from 62.08 to 82.54% and from 66.19 to 79.03% respectively for maceration and aqueous decoction after 15 minutes of reaction. . Meanwhile, in the ethanolic medium, the percentage of inhibition ranges from 88.27 to 90.55%. The inhibition percentages at 20 mM and 40 mM CTAB are substantially the same and it can be concluded that 20 mM CTAB are sufficient to carry out the reaction.



de: decoction; aq: aqueous

Figure 7: Kinetics of inhibition of DPPH by plant extracts

Meanwhile, the aqueous macerate of *B. africana* induced an inhibition ranging from 66.43 to 77.87% for CTAB concentrations ranging from 2 to 40 mM. In aqueous medium, the percentage inhibition is 88.79%. The decoction offers the best percentages of inhibition which are 71.71%, 85.56% and 87% respectively for 2 mM, 20 mM and 40 mM of CTAB. In ethanolic medium, the percentage inhibition is very high, a value of 94.62%. As before, 20 mM CTAB is sufficient to have good inhibition.

As far as the extracts of *M. paniculata* are concern, their exhibition behavior is quite different from the other plant extracts studied. In fact the inhibition progressively evolves towards the plateau which is generally weak compared to other plant extracts. Thus, for the aqueous extract, the inhibition increases from 63.95 to 73.74% (plateau) when passing from 2 to 40 mM whereas in the ethanolic medium, the percentage inhibition is 67.30%. .

Inhibition in the presence of *M. paniculata* decoction is even weaker. As the CTAB concentration was increased from 2 to 40 mM, the inhibition increased from 45.9 to 63.32%. In ethanolic medium, the inhibition is 66.19%. For the two extracts studied, 20 mM CTAB is enough to have the best inhibition

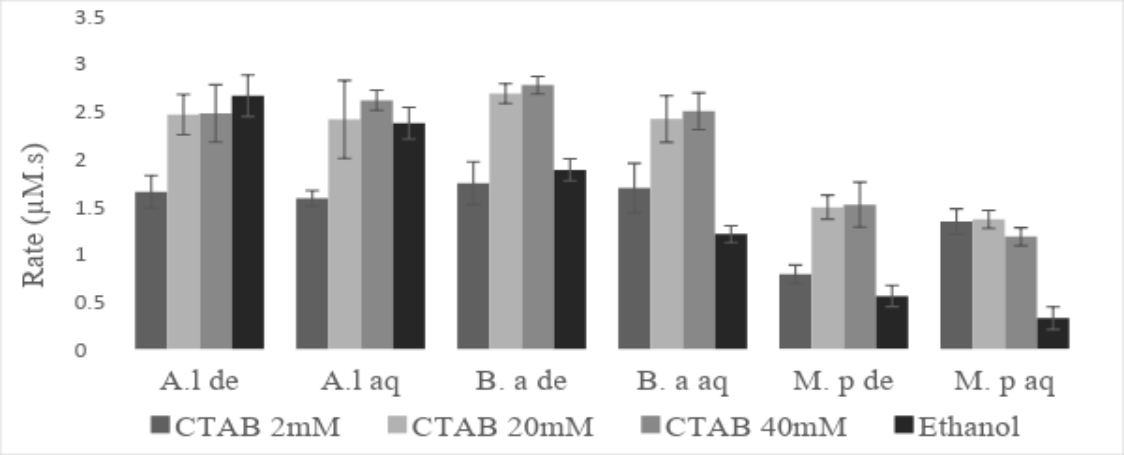
Kenetic study of DPPH reduction by plant extract

The study of the kinetics of the reaction following the method used for gallic acid allowed us to determine the reaction rates after 15 seconds (figure 8).

The rate of disappearance of the DPPH increases with the increase of the concentration of CTAB. The reaction rates at 20 mM and 40 mM CTAB are substantially the same and close to those in ethanol. Compared with gallic acid, it is retained that at 20 mM and 40 mM CTAB, the extracts of *A. leiocarpa*, prepared at 1 mg/mL have a speed close to that of gallic acid at 100 µM or $16.8.10^{-3}$ mg/mL.

The reaction rates recorded graphic 2 are comparable to those of *A. leiocarpa* extracts and the same conclusions can be drawn. However, it can be seen that the speeds in ethanolic medium are lower than those in micellar medium.

Whether micellar or ethanol, the rates of disappearance of DPPH are low compared to the other two plant extracts (graphic 2). This could be the basis of the low percentages of inhibition obtained after 15 minutes of reaction. The explanation lies in the fact that the chemical compositions of these plants are not the same because the interactions between antioxidants and DPPH depend on the number of hydroxyl groups present in the molecules as well as their structural conformation [29]. Indeed, *M. paniculata* is a plant very rich in coumarin in view of the strong fluorescence that it gives to UV 365 nm while the other two plants do not contain it and coumarins are less rich in hydroxyl groups than phenolic compounds whose presence has been clearly demonstrated in *A. leiocarpa* and *B. africana*. The author [10] confirm this remark in that their work has shown that the polyphenolic compounds among which gallic acid have a greater antiradical activity than the mono-phenol compounds of which the acid *p*-coumarique.



dec: decoction; aq: aqueous

Figure 8: Rate of disappearance of DPPH in the presence of plant extracts

Conclusion and perspectives

This study has allowed us to explore a new method of studying anti-radical activity with DPPH, which is increasingly used by researchers around the world. The originality and importance of this method is well established because it effectively shows the behavior of the antioxidant in the face of free radicals in the human biological environment. In fact, the conventional method of DPPH is carried out only in ethanolic or methanolic medium while the new method is carried out in an aqueous emulsifier medium, here the CTAB. The presence of CTAB significantly improves the percentage of inhibition and the results are often better than those obtained in ethanolic medium. In view of the results obtained, extracts of *A. leiocarpa* and *B. africana* can be used to effectively fight against oral infections, including periodontitis and caries, one of the causes of which is the presence of free radicals in the body oral cavity. This work has been carried out in acidic environment and it will be interesting to repeat the same experiment in slight basic medium using another micellar medium in order to compare the results knowing acid medium in favorable to oral diseases.

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