International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 5 No. 2 September 2017, pp. 71-75

# *Comparative Study of the Composition of Aqueous Extracts of Green Tea (Camellia Sinensis) in Total Alkaloids, Total Flavonoids, Total Polyphenols and Antioxidant Activity with the Leaves of Combretum Glutinosum, Combretum Micranthum and the Red Pulps of Hibiscus Sabdariffa.*

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***Abstract* — Green tea (Camellia sinensis) is by far the most consumed beverage in the world after water. This consumption is mainly due to its antioxidant properties and its secondary metabolites richness. In Senegal, tea is consumed, but also herbal teas, including Combretum glutinosum, Combretum micranthum, for breakfast or Hibiscus sabdariffa, as a drinking juice. The purpose of this study is to compare Green tea with these herbal teas in term of secondary metabolites content and antioxidant activity. The plant material used consists of leaves Camellia sinensis, Combretum glutinosum, Combretum micranthum, and red pulp of Hibiscus sabdariffa. These different parts of plants were dried and then ground into fine powder before being extracted by infusion and then concentrated the infuser in a dry rotary evaporator. The dry extracts are used for the quantitative determination of alkaloids, flavonoids, polyphenols and the antioxidant activity by the ABTS method. On the different plant extracts tested, the leaves of Combretum glutinosum are richer than all the other plants studied here in terms of alkaloids, flavonoids and polyphenols with concentrations of 6.96 µg/mg, 134.94 µg/mg and 374.50 µg/mg of dry extracts. In flavonoids, leaves of C. Gutinosum are followed by the red pulp of Hibiscus sabdariffa with 1.626 µgQE/mg. The latter are also the poorest in flavonoids and polyphenols compared to Camellia sinensis and leaves of C. Micranthum with respective concentrations of 107.90µgQE/mg and 97.70 µgQE/mg in flavonoids and 258.50 µgGAE/mg 223.50 µgGAE/mg in polyphenols. With regard to the antioxidant activity, C. Glutinosum are most active with an IC50 of 6.101 µg/ml followed by tea with 6.713 µg/ml at the moment when C. Micranthum and Hibiscus sabdariffa had respectively IC50s of 6.727 and 11.76 µg/ml.**

***Keywords* — *Alkaloids; Flavonoids; Polyphenols; Antioxidant.***

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## I. INTRODUCTION

Green tea (Camellia sinensis) is the most consumed beverage in the world after water so that the way it is prepared is variable. This large consumption is due primarily to its antioxidant properties, to its richness in certain secondary metabolites, and to the pharmacological effects attributed to it [1].

In Senegal, tea is also popular among the population, which is generally imported from Asia. Besides these green leaves of Camellia sinensis are consumed other herbal teas such as the leaves of Combretum glutinosum, Combretum

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micranthum, for the breakfast in general or the red pulp of Hibiscus sabdariffa, like juice of drink. These latter are native, available, affordable, and grow naturally without any toxic substances (pesticides or fungicides).

However, some of these local plants have similar activities to those of tea such as antibacterial with Combretum glutinosum [2] antioxidant with Combretum micranthum [3] or with antiviral Hibiscus sabdariffa. [4]

The purpose of this study is to determine some of the secondary metabolites present in these plants such as total alkaloids, total flavonoids, total polyphenols and also to assess their antioxidant activities in order to compare these native plants with green tea.

## II. MATERIALS AND METHODS

### A. Plant material

The plant material used consists of green leaves of Combretum glutinosum, Combretum micranthum, Camellia sinensis and red pulp of Hibiscus sabdariffa.

### B. Preparation of plant material

The leaves of Combretum glutinosum, Combretum micranthum, Camellia sinensis and the red pulps of Hibiscus sabdariffa used in this study were harvested. The green tea leaves used are purchased commercially. These different samples were dried and then ground into fine powder.

### C. Extraction

Test portions of 30 g of leaf powder or plant pulp were infused with 300 ml of water at 90 ° C until cooling. The mixture was filtered and the infused were concentrated to dryness in a rotary evaporator.

These solids were used for the tests.

### D. Dosage of total alkaloids

The alkaloids were dosed according to the method described by Sreevidya. A test sample of 5 ml of 5 mg/ml extract solution of previously prepared concentration in a pH aqueous solution maintained at 2-2.5 with diluted HCl and then a 2 ml quantity of Dragendorff (DR) reagent a were added and the precipitate formed were centrifuged. The centrifugate was checked for complete precipitation by adding DR. After centrifugation, the centrifugate was decanted completely and meticulously. The precipitate was further washed with alcohol. The filtrate was discarded and the residue was then treated with 2 ml disodium sulfide solution.

The brownish black precipitate formed were then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulfide. The residue was dissolved in 2 ml concentrated nitric acid, with warming if necessary. This solution was diluted to 10mL in a standard flask with distilled water; 1 ml were then pipetted out, and 5 ml thiourea solution were added to it. The observance was measured at 435 NM against the blank containing nitric acid and thiourea. A calibration curve was carried out in parallel under the same operating conditions using bismuth nitrate and the results are expressed in milligram µg/mg of dry plant extract. The amount of bismuth present in the solution was calculated by multiplying the absorbance values with the factor, taking suitable dilution factor into consideration [5].

### E. Dosage of total flavonoids

The total flavonoid content of each plant extracts determined by a colorimetric method. 0.5 ml of the aqueous extract solution at 1 mg/ml concentration were mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO2 solution (15%). After 6 min, 0.15 ml of aluminum chloride (AlCl3) solution (10%) was added and allowed to stand for 6 min, then 2 ml of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture were thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as quercetin equivalent (µg quercetin/mg dried extract).

### F. Dosage of total polyphenols

The total polyphenols content of each plant extract were determined with the Folin–Ciocalteu's reagent (FCR) according to the published method [7]. 0.1 ml of aqueous extract solution at 1 mg/ml concentration were mixed with 2 ml of a freshly prepared 2% Na2CO3, the whole were stirred by a vortex [7]. After five minutes, 100 µl of the Folin-Ciocalteu reagent (1 N) were added to the mixture, the whole were left for 30 minutes of incubation at room temperature and the reading is performed against a blank. The absorbance was then measured at 700 nm. Results were expressed as gallic acid equivalent (µg gallic acid/mg dried extract).

### G. *Experimental protocol of the ABTS test*

A quantity of 38.40 mg of ABTS was dissolved beforehand in 10 ml of water.

A quantity of 6.75 mg of potassium persulfate is added thereafter. The mixture obtained were kept in the dark and at ambient temperature for 12 hours before use. This mixture was diluted with ethanol in order to obtain an absorbance of the order of 0.7 to 734 nm.

The antioxidant activity was measured by mixing 0.8 ml of the extract dissolved in ethanol with 3.2 ml of the ABTS solution in order to obtain the concentrations varying from 1 to 10 µg/ml.

Ascorbic acid, used as reference antioxidants, was dissolved in ethanol and tested at the same concentrations. The absorbance reading was made after 2 minutes at the spectrophotometer at 734 nm using ethanol as blank. Three absorbance measurements were performed for each concentration [8].

The antioxidant activity was denominated as the percentage inhibition (PI) of the absorbance of the radical which corresponds to:

$$PI = \frac{A0 - A1}{A0} x100$$

A0 : absorbance of the solution of ABTS
A1: absorbance of the solution of ABTS after addition of the extract tested to a given concentration and after the reaction.

IC50 values are calculated from GraphPad Prism software (v5.0d, San Diego, CA) using a non-linear regression model using percent inhibition (PI) values.

## III. Results

### A. *Results of dosage tests of total alkaloids, total flavonoids and total polyphenols.*

The following table shows the results of secondary metabolite testing in plant samples.

TABLE 1: Quantities of alkaloids, flavonoids and polyphenols µg/mg of plant extracts

| Quantité | Total alcaloids [µg/mg] | Total flavonoids [µgQE/mg] | Total polyphenol [µgAGE/mg] |
|---|---|---|---|
| *Camellia sinensis* | 0.654 | 107.9 | 258.5 |
| *C. glutinosum* | 6.96 | 134.94 | 374.5 |
| *C. micranthum* | 1.114 | 97.7 | 223.5 |
| *Hibiscus sabdariffa* | 1.626 | 24.00 | 105.5 |

µg/mg: microgram of bismuth nitrate equivalent per milligram; µgQE/mg: microgram equivalent quercetin per milligram; µgAGE/mg: microgram equivalent gallic acid per milligram.

### B. *Results of antioxidant activity tests*

The following table gives the inhibition percentages of the various extracts tested by ABTS method

TABLE 2 : PERCENT INHIBITION OF EXTRACTS OF LEAVES AND PULPS TESTED WITH ABTS

| Concentrations µg/ml | *Camellia sinensis* | *Combretum glutinosum* | *Combretum micranthum* | *Hibiscus sabdariffa* |
|---|---|---|---|---|
| 1 | 19.554 | 12.160 | 14.655 | 2.015 |
| 2 | 20.691 | 26.694 | 19.124 | 2.631 |
| 3 | 25.833 | 32.884 | 25.381 | 3.832 |
| 4 | 32.382 | 44.228 | 35.003 | 5.736 |
| 5 | 38.932 | 69.422 | 43.324 | 6.144 |
| 6 | 58.762 | 71.584 | 43.886 | 7.542 |
| 7 | 68.506 | 78.978 | 52.185 | 9.260 |
| 8 | 70.503 | 80.876 | 52.593 | 10.560 |
| 9 | 77.941 | 98.036 | 61.344 | 12.090 |
| 10 | 90.521 | 99.261 | 63.010 | 12.607 |

Table 3 gives the IC50 of the various extracts calculated with the GraphPad Prism software.

TABLE 3 : IC50 OF EXTRACTS OF LEAVES AND PULPS TESTED BY ABTS METHOD

| Extraits | *Camellia sinensis* | *Combretum glutinosum* | *Combretum micranthum* | *Hibiscus sabdariffa* |
|---|---|---|---|---|
| $CI_{50}$ µg/ml | 6.713 | 6.101 | 6.727 | 11.76 |

The following table gives the percentages of inhibition of the ascorbic acid tested with ABTS and Table 5 its IC50

TABLE 4 : PERCENTAGE OF INHIBITION AND IC50 OF ASCORBIC ACID TESTED WITH ABTS

| Concentrations µg/ml | % Inhibition |
|---|---|
| 1 | 31.467 |
| 2 | 60.483 |
| 3 | 77.367 |
| 4 | 78.456 |
| 5 | 98.311 |
| 6 | 99.558 |

TABLE 5 : IC50 OF ASCORBIC ACID TESTED BY ABTS

| Molécule de référence | Acide ascorbique |
|---|---|
| $CI_{50}$ µg/ml | 1.83 |

## IV. Discussion

Green tea is mainly consumed, because of its pharmacological effects but also because of its richness in secondary metabolites. Many scientific studies have shown that the pharmacological effects of plants observed on the human body are due to the presence of secondary metabolites [1].

These secondary metabolites present in plants can be assayed by several methods including the colorimetric method using the UV spectrophotometer.

Thus, for the quantitative determination of alkaloids, the leaves of Combretum glutinosum are the richest with a concentration of 6.96µg/mg of extracts, followed by extracts of the Hibiscus sabdariffa pulp (1.626µg/mg) and leaves of Combretum micranthum (1.114 µg/mg) at the time when the green leaves of Camellia sinensis have an amount of 0.654 µg/mg (Table 1).

For the flavonoid assay, the leaves of Combretum glutinosum were the richest with 134.94 µgQE/mg followed by green leaves of Camellia sinensis 107.9 µgQE/mg and Combretum micranthum with a concentration of 97.7 µgQE/mg. Among the samples, Hibiscus sabdariffa red pulps are the lowest in flavonoids with a quantity of 24.00µgQE/mg extract (Table 1).

Quantification of polyphenols showed that the leaves of Combretum glutinosum remained the richest with a concentration of 374.5 µgAGE/mg followed by green leaves of Camellia sinensis, which contained 258.5 µgAGE/mg, and then followed closely by the leaf extract Of Combretum micranthum with a concentration of 223.5 µgAGE/mg. The red pulps of Hibiscus sabdariffa are the least rich with an amount of 105.5 µgAGE/mg. (Table 1).

At the dosages of these different secondary metabolites, the leaves of Combretum glutinosum are the richest in alkaloids, flavonoids and polyphenols, followed on both sides by the leaves of Camellia sinensis and Combretum micranthum. These groupings of molecules can be responsible for different activities, including the antioxidant activity [10].

Evaluation of the antioxidant activity was carried out by the free radical, ABTS colorimetric method.

Among the four plants tested, the leaves of Combretum glutinosum were most active with an IC50 of 6.101 µg/ml, followed by leaves Camellia sinensis and Combretum micranthum which had respective IC50 values of 6.727 µg/ml and 6.713 µg/ml . Hibiscus sabdariffa red pulps were the least active with an IC50 of 11.76 µg/ml (Tables 2 and 3). The ascorbic acid taken as controls were more active than the plant extracts with an IC50 of 1.830 µg/ml.

These activities are proportional to the quantity of polyphenols present in plant extracts. The antioxidant activity is often attributed to the presence of polyphenols [11].

Based on these results, the green tea consumed for most of the time, among others because of its antioxidant activity, or by its secondary metabolites richness, the leaves of Combretum glutinosum have proved ten times richer in alkaloids than green tea, more than 20% in flavonoids and more than 30% in polyphenols. Currently world tea trade reached more than $ 13 billion in 2013 [12].

## V. Conclusion

These interesting results show the richness of some of our plants in secondary metabolites and antioxidants, including Combretum glutinosum compared to green tea and thereby give validation to the use of these herbal teas. Moreover, they also show that the exploitation of Senegalese flora can serve as a source of new biomolecules to fight against cardiovascular diseases and also contribute to Senegalese economic growth through its commercialization.

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