

Polyphenols Composition and Antioxydant Capacity of Herbal Tea Composite *Citrus sinensis* Peel and *Moringa oleifera* Leaf

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How to cite this paper: Koffi, K.A., Assa, R.R., Konan, A.G. and Fofie, N.B.Y. (2025) Polyphenols Composition and Antioxydant Capacity of Herbal Tea Composite *Citrus sinensis* Peel and *Moringa oleifera* Leaf. *Food and Nutrition Sciences*, 16, 996-1010. <https://doi.org/10.4236/fns.2025.168057>

Received: March 4, 2024

Accepted: August 24, 2025

Published: August 27, 2025

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Abstract

This study aimed to determine the polyphenolic composition, to evaluate the antioxydant activity of composite teas of *Citrus sinensis* peel and *Moringa oleifera* leaves. Samples of zest and moringa collected were processed. The phenolic composition as well as the antioxydant capacity was determined on eleven composite powders from the composite central plane. The total polyphenol content was determined by the Folin-Ciocalteu method, while the flavonoid content was assessed by the aluminum chloride method. Antioxydant capacity was determined by the DPPH free radical reduction method (1,1-diphenyl-2-picrylhydrazyl) and by the iron reducing method (FRAP). The herbal teas obtained have a high content of phenolic compounds. In fact, the herbal tea consisting of (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) obtained the highest polyphenol content, while EC10 (70% *Citrus sinensis*/30% *Moringa oleifera*) has the highest flavonoid content. EF01 (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) has the highest reducing activity, however, EF01 has a strong anti-free radical activity. There is a strong correlation between. These composite teas are a good source of total polyphenols and flavonoids. They also have good antioxydant properties.

Keywords

Polyphenols, Antioxydants, Herbal Tea, *Citrus sinensis*, *Moringa oleifera*, Côte d'Ivoire

1. Introduction

Today, herbal teas and tea are the most consumed beverages in the world after water [1]. In fact, the Dutch government recommends the consumption of three cups of tea per day based on the decreased risk of stroke and hypertension.

In South Africa, rooibos tea is widely available. In Côte d'Ivoire, people buy *Camellia sinensis* tea in supermarkets and stores, but some buy it from street vendors. Herbal teas, meanwhile, are traditionally made and sold in markets. Several epidemiological studies [2] [3] establish a relationship between herbal tea consumption and the incidence of various chronic diseases recognized to be associated with oxidative stress due to the antioxidant effects of phenolic compounds [4] [5]. *Camellia sinensis*, *Artemisia annua*, *Moringa oleifera*, *hibiscus sabdarriâ* and *Citrus sinensis* are also renowned for their polyphenol content [6]. Indeed, *Citrus sinensis* peels are high in total polyphenols and vitamin C [7]. *Moringa oleifera* herbal teas are also rich in polyphenolic compounds, notably flavonoids, certain minerals (Ca, P, Fe) and essential amino acids [8] [9]. These compounds confer them several biological activities including antibacterial, anticarcinogenic, anti-inflammatory, antiviral, antiallergic, estrogenic and immunostimulant [10]. However, in Côte d'Ivoire, *Citrus sinensis* orange peel is discarded in the streets, constituting a source of environmental pollution.

Thus, in the present work, a composite herbal tea was formulated from *Citrus sinensis* zest (epicarp) and *Moringa oleifera* leaves. The aim is to determine the polyphenolic composition of these teas and assess their antioxidant activity.

2. Materials

2.1. Biological Materials

The biological material used in this study consisted of treated *Citrus sinensis* zest and *Moringa oleifera* leaves.

2.2. Technical Material

Weighing was carried out on a weighing balance (Shimadzu, Japan) and a precision balance (OSI, M-220 D). A binatone blender (BLG 555, China) was also used to grind the samples, which were then sieved using commercial sieves (mesh \leq 100 μm). Humidity was determined using an oven (memmert UL 30, Germany). Wavelengths were read using a spectrophotometer (Jenway 7315, UK).

3. Methods

3.1. Sample Collection

The samples were collected in the city of Abidjan, in the south of Côte d'Ivoire. *Citrus sinensis* peels were collected in the communes of Adjamé (the biggest citrus market, particularly for oranges), Cocody (a wealthy commune) and Yopougon (the biggest commune, middle class).

Next, 10 kg of fresh *Moringa oleifera* leaves were collected from gardeners in the commune of Bingerville. After collection, the samples were transported to the

laboratory for further study. Samples were collected in triplicate.

3.2. Powder Production Technology

The collected samples were sorted, soaked in distilled water for 5 min and then drained. They were then withered at 50°C for 6 h, oxidized for 4 h and dried at 50°C to constant mass. Finally, each species was ground.

3.3. Preparing Composite Powders

The powders obtained from each species were mixed using a composite central plane. In this way, formulations with different proportions were determined. This experimental design took into account two variables (quantity of *Citrus sinensis* zest and quantity of *Moringa oleifera* leaves). The combination of the 2 variables led to 11 formulations with 4 factorial trials (FT), 4 star trials (ST) and 3 center trials (CT). The various composite powders, the reference tea and their codes are shown in **Table 1** [11].

Table 1. Different composite powders for herbal teas and their codes.

Formulation code	Quantity of powder (%)	
	<i>Citrus sinensis</i> peel (%)	<i>Moringa oleifera</i> (%)
EF01	77.89	22.11
EF02	84.14	15.85
EF03	55.85	44.14
EF04	65.59	34.4
EE05	62.5	37.5
EE06	75	25
EE07	87.5	12.5
EE08	58.33	41.66
EC09	70	30
EC10	70	30
EC11	70	30
ET	Control	

EF: factorial essay; EE: star essay; EC: essay at the centre; ET1. sample control.

3.4. Preparing Herbal Teas

Herbal teas were prepared according to [12]. In addition, 100 ml of boiling water was poured over 2 g of each composite powder, then filtered after 5 min.

3.5. Determination of Herbal Tea Composition

3.5.1. Total Polyphenol Content

Total polyphenol content was determined using the Folin-Ciocalteu method [13]. Absorbance was measured at a wavelength of 725 nm. Gallic acid was used as a standard. Results were expressed as milligram equivalent of gallic acid per 100 g of dry matter (mg EAG/100g MS) and milligram equivalent of gallic acid per liter

of herbal tea (mg EAG/L).

3.5.2. Flavonoid Content

Flavonoid content was determined according to [14]. To 0.5 mL infusion were added 0.5 mL distilled water, 0.5 mL 10% (w/v) aluminum chloride, 0.5 mL 1M sodium acetate and 2 mL distilled water. After reaction in the dark for 30 min, optical density was read at 415 nm. Results were expressed in milligram equivalents of quercetin per 100 g of dry matter and in milligram equivalents of quercetin per liter of herbal tea.

3.5.3. Anthocyanin Content

1 mL extract was diluted in 1.5 mL pH 1.0 buffer and 1.5 mL pH 4.5 buffer respectively. The absorbance (A) of these two solutions was read at $\lambda_{\max} = 520$ nm and $\lambda_{\max} = 700$ nm respectively [15]. Total anthocyanin (TA) content was expressed in milliequivalents of cyanidin 3-glycoside per 100 g of dry matter and in milliequivalents of cyanidin 3-glycoside per liter of herbal tea.

3.5.4. Assessment of Antioxydant Activity

Two methods were used to determine the antioxydant activity of herbal teas: DPPH (1,1-diphenyl-2-picrylhydrazyl), et FRAP (Ferric reducing antioxydant power).

1) DPPH

The free radical scavenging activity of herbal teas was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical reduction method described by [16]. A 3 mM DPPH solution was prepared. 0.5 mL of herbal tea was added to 1.5 mL of DPPH solution. After incubation for 30 min in the dark, absorbance was measured at 517 nm using the DPPH solution as control. Assays were performed in triplicate. Results were expressed in equivalent micromoles of ascorbic acid per gram of dry matter and in equivalent micromoles of ascorbic acid per 100 mL of herbal tea.

2) FRAP

The reducing power of an extract is associated with its antioxydant power. The iron activity of our extracts was determined according to the method described by [16]. based on the chemical reduction of Fe^{3+} present in the $\text{K}_3\text{Fe}(\text{CN})_6$ complex to Fe^{2+} . A 1 ml volume of infusion was mixed with 2.5 ml of 0.2 M phosphate buffer (pH = 6.6) and 2.5 ml of 1% $\text{K}_3\text{Fe}(\text{CN})_6$ solution. Incubate at 50°C for 20 minutes, then cool to room temperature. Next, 2.5 ml of 10% trichloroacetic acid was added to stop the reaction. 2.5 ml of the extract was added to 2.5 ml of distilled water, and 500 μl of 0.1% ($\text{FeCl}_3, 6\text{H}_2\text{O}$) solution was added to the mixture.

Absorbances were read against a blank at 700 nm using a spectrophotometer. Trials were carried out in triplicate. Results were expressed in micromoles of ascorbic acid equivalent per gram of dry matter ($\mu\text{mol EAA/gMS}$) and in micromoles of ascorbic acid equivalent per 100 millilitres of herbal tea ($\mu\text{mol EAA}/100\text{mL}$).

3) Composite antioxydant index

The antioxydant composite index (ACI) was determined according to [4] and

calculated as follows:

$$ACI = \frac{\text{sample score}}{\text{best score}} * 100$$

4. Results and Discussion

4.1. Results

Table 2 shows the polyphenol, total flavonoid and anthocyanin contents of the samples. Polyphenol contents range from 645.41 to 2961.38 mg EAG/100g MS, *i.e.* from 129.08 to 592.28 mg EAG/L infusion. These levels differed statistically ($p \leq 0.05$) between the different samples. The control sample (ET) made up of 100% *Camellia sinensis* had the highest polyphenol content (2961.38 mg EAG/100 g), while (EE05) made up of 62.5; 37.5% *Citrus sinensis* and *Moringa oleifera* respectively had the lowest total polyphenol content (645.41 mg EAG/100g). Infusions of EC09, EC10, EC11 have identical total polyphenol contents ($p > 0.05$). These contents are estimated at 734.82 mg EAG/100g or 146.99 mg EAG/L infusion. Moreover, these have the same composition: 70% *Citrus sinensis* and 30% *Moringa oleifera*.

Table 2. Total polyphenol and flavonoid content of various composite herbal teas.

herbal tea	Total polyphenol content (TPC)		Total flavonoid content (TFC)		Anthocyanin content (AT)	
	mg EAG/L	mg EAG/100g DW	mg EQ/L	mgEQ/100g DW	meq/100g	meq/L
EF01	150.42 ± 2.66b	752.12 ± 13.3b	70.94 ± 1.25c	354.72 ± 6.23c	2.42 ± 0.59a	0.48 ± 0.11a
EF02	141.87 ± 1.73cd	709.35 ± 8.63cd	58.37 ± 1.52f	291.83 ± 7.59f	0.62 ± 0.29b	0.12 ± 0.05b
EF03	145.76 ± 0.15bc	728.79 ± 0.75bc	72.82 ± 1.38c	364.09 ± 6.90c	0.87 ± 0.05b	0.17 ± 0.01b
EF04	140.40 ± 1.82d	702.00 ± 9.1d	64.16 ± 1.14de	320.78 ± 5.69de	0 ± 0.00b	0 ± 0.00b
EE05	129.08 ± 1.8e	645.41 ± 8.98e	61.65 ± 0.93def	308.25 ± 4.64def	0.75 ± 0.47b	0.15 ± 0.09b
EE06	129.43 ± 1.33e	647.14 ± 6.65e	60.73 ± 1.47ef	303.63 ± 7.33ef	0.7 ± 1.00b	0.14 ± 0.2b
EE07	141.26 ± 0.02cd	706.32 ± 0.03cd	60.82 ± 0.04ef	304.11 ± 0.21ef	1.00 ± 1.41ab	0.2 ± 0.28ab
EE08	139.36 ± 2.77d	696.82 ± 13.86d	65.18 ± 0.81d	325.89 ± 4.06d	0.41 ± 0.59b	0.08 ± 0.11b
EC09	146.97 ± 2.33b	734.84 ± 11.66b	76.78 ± 1.14b	383.91 ± 5.69b	0.57 ± 0.00b	0.11 ± 0.00b
EC10	146.98 ± 2.33b	734.86 ± 11.65b	76.80 ± 1.13b	383.93 ± 5.68b	0.58 ± 0.82b	0.12 ± 0.16b
EC11	146.99 ± 2.33b	734.82 ± 11.66b	76.80 ± 1.14b	385.91 ± 5.69b	0.55 ± 0.35b	0.11 ± 0.07b
ET	592.28 ± 5.94a	2961.38 ± 29.7a	91.26 ± 5.86a	456.29 ± 29.28a	1.00 ± 0.35ab	0.2 ± 0.07ab

Results are means ± S.D. (n = 3); values of the same column, followed by the same letter (a-f) are not statistically different as measured by Duncan's test ($p > 0.05$). EF: factorial essay; EE: star essay; EC: essay at the centre; ET1. sample control; EQ: quercetin equivalent; EAG: gallic acid equivalent ET. sample control; Composite powder with respective percentages of *Citrus sinensis* and *Moringa oleifera*: EF01. 77.89/22.11; EF02. 88.14/15.85; EF03. 55.85/44.14; EF04. 65.59/34.4; EE05. 62.5/37.5; EE06. 75/25; EE07. 87.5/12.5; EE08. 58.33/41.66; EC09. 70/30; EC10. 70/30; EC11. 70/30.

The total flavonoid content of the composite herbal teas ranged from 291.83 to 383.93 mg EAG/100g, representing 58.37 to 76.80 mg EQ/L infusion. The EF02 trial (88.14% *Citrus sinensis* and 15.86% *Moringa oleifera*) contained the lowest flavonoid content, while EC10 (70% *Citrus sinensis* and 30% *Moringa oleifera*)

contained more flavonoids. However, the sample made up of 100% *Camellia sinensis* (ET) has the highest content ($p \leq 0.05$).

The anthocyanin content of the various samples ranged from 0 to 0.04 mg/100mL infusion, or 0 to 0.24 mg/100g dry matter. Among the infusions, EF01 (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) has the highest anthocyanin content, while EF04 (65.59% *Citrus sinensis*/4.4% *Moringa oleifera*) and EC09 (70% *Citrus sinensis*/30% *Moringa oleifera*) have the lowest. EF02, EF03, EF04, EE05, EE06, EE08, EC09, EC10 and EC11 have identical anthocyanin contents ($p > 0.05$).

4.2. Identify the Headings

Antioxydant capacity

The antioxydant activity of the infusions was determined using two tests (DPPH and FRAP). The values obtained are shown in **Table 3**. The control sample (ET) made from 100% *Camellia sinensis* showed the highest free radical scavenging activity ($170.10 \pm 0.17 \mu\text{mol EAA/g MS}$), *i.e.* $340.21 \pm 0.33 \mu\text{mol EAA/100mL}$ infusion, as well as the highest reducing capacity ($501.59 \pm 2.2 \mu\text{mol EAA/g DW}$). The composite herbal tea from EF01 (77.89% *Citrus sinensis* and 22.11% *Moringa oleifera*) has an antiradical activity close to that of the control (ET). This activity is estimated at $149.32 \mu\text{mol EAA/g MS}$ or $298.65 \mu\text{mol EAA/100mL}$ herbal tea. By contrast, EE07 (87.5% *Citrus sinensis*; 12.5% *Moringa oleifera*) and EE06 (75% *Citrus sinensis*; 25% *Moringa oleifera*) have the lowest free radical scavenging activity, estimated at 204.44 and $122.5 \mu\text{mol EAA/100mL}$ respectively, at the same 20 mg/mL concentration.

Table 3. Antioxydant capacity of various composite herbal teas.

Herbal tea	Antioxydant capacity			
	DPPH ($\mu\text{mol EAA/gMS}$)	DPPH ($\mu\text{mol EAA/100mL}$)	FRAP ($\mu\text{mol EAA/g MS}$)	FRAP ($\mu\text{mol EAA/100mL}$)
EF01	$149.32 \pm 0.17\text{b}$	$298.65 \pm 0.33\text{b}$	$93.14 \pm 2.75\text{ef}$	$186.29 \pm 5.51\text{ef}$
EF02	$122.88 \pm 0.5\text{f}$	$245.76 \pm 1.00\text{f}$	$93.65 \pm 0.08\text{ef}$	$187.30 \pm 0.17\text{ef}$
EF03	$135.63 \pm 0.83\text{d}$	$271.26 \pm 1.67\text{d}$	$110.31 \pm 0.88\text{b}$	$220.63 \pm 1.77\text{b}$
EF04	$138.93 \pm 0.17\text{c}$	$277.87 \pm 0.33\text{c}$	$95.47 \pm 4.62\text{de}$	$190.95 \pm 9.25\text{de}$
EE05	$129.25 \pm 0.83\text{e}$	$258.51 \pm 1.67\text{e}$	$92.26 \pm 0.44\text{ef}$	$184.53 \pm 0.88\text{ef}$
EE06	$61.25 \pm 0.17\text{h}$	$122.50 \pm 0.33\text{h}$	$89.50 \pm 0.26\text{f}$	$179.00 \pm 0.53\text{f}$
EE07	$102.22 \pm 0.33\text{g}$	$204.44 \pm 0.67\text{g}$	$84.09 \pm 0.26\text{g}$	$168.18 \pm 0.53\text{g}$
EE08	$123.94 \pm 1.34\text{f}$	$247.88 \pm 2.67\text{f}$	$83.46 \pm 0.62\text{g}$	$166.92 \pm 1.24\text{g}$
EC09	$138.11 \pm 1.00\text{c}$	$276.22 \pm 2.00\text{c}$	$99.81 \pm 0.8\text{c}$	$199.63 \pm 1.6\text{c}$
EC10	$138.46 \pm 0.83\text{c}$	$276.93 \pm 1.67\text{c}$	$99.80 \pm 1.06\text{c}$	$199.60 \pm 2.13\text{c}$
EC11	$138.34 \pm 0.33\text{c}$	$276.69 \pm 0.67\text{c}$	$99.82 \pm 1.51\text{c}$	$199.64 \pm 3.02\text{c}$
ET	$170.10 \pm 0.17\text{a}$	$340.21 \pm 0.33\text{a}$	$501.59 \pm 2.22\text{a}$	$1003.19 \pm 4.44\text{a}$

Results are means \pm S.D. ($n = 3$); values of the same column. followed by the same letter (a-h) are not statistically different as measured by Duncan's test. EF: factorial essay; EE: star essay; EC: essay at the centre; ET. sample control; Composite powder with respective percentages of *Citrus sinensis* and *Moringa oleifera*: EF01. 77.89/22.11; EF02. 88.14/15.85; EF03. 55.85/44.14; EF04. 65.59/34.4; EE05. 62.5/37.5; EE06. 75/25; EE07. 87.5/12.5; EE08. 58.33/41.66; EC09. 70/30; EC10. 70/30; EC11. 70/30.

FRAP test values range from 166.92 $\mu\text{mol EAA}/100\text{mL}$ herbal tea, equivalent to 83.46 $\mu\text{mol EAA}/\text{g MS}$ (EE08: 58.33% *Citrus sinensis* 41.66% *Moringa oleifera*) to 220.63 $\mu\text{mol EAA}/100\text{mL}$, equivalent to 110.31 $\mu\text{mol EAA}/\text{g MS}$ (EF03: 55.85% *Citrus sinensis*; 44.14% *Moringa oleifera*). Herbal teas EC09, EC10, EC11 made from 70% *Citrus sinensis*/30% *Moringa oleifera* show the same reducing activity ($p > 0.05$). Also, EF01 (77.89% *Citrus sinensis*; 22.11% *Moringa oleifera*), EF02 (88.14% *Citrus sinensis*; 22.11% *Moringa oleifera*), EE05 (62.5% *Citrus sinensis*; 37.5% *Moringa oleifera*) are not statistically different ($p > 0.05$).

Antioxydant Composite Index

Table 4 shows the antioxydant composite index (ACI) of the different infusions. Indeed, the reference sample ET (*Camellia sinensis*) has the highest index. The ICA of the composite herbal teas is ranked in ascending order as follows: EF01 > EF03 > EC10 > EC11 > EC09 > EF04 > EE05 > EF02 > EE08 > EE07 > EE06. Herbal tea EF01 (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) has the highest composite antioxydant index, followed by EF03 (55.85% *Citrus sinensis*/44.14% *Moringa oleifera*) and EC10 (70% *Citrus sinensis*/30% *Moringa oleifera*). These indices are 53.2, 50.9 and 50.6 respectively. Furthermore, EE08 (58.33% *Citrus sinensis*/41.66% *Moringa oleifera*); EE07 (87.5% *Citrus sinensis*/12.5% *Moringa oleifera*); EE06 (75% *Citrus sinensis*/25% *Moringa oleifera*) present the lowest ICA. They are estimated at 44.8, 38.4 and 26.9 respectively.

Table 4. Antioxydant potency composite index of the tested samples calculated from two antioxydant capacity measurements scaled to relative percentages.

Tisanes	index dpsh	index frap	Antioxydant composite index (ACI)
EF01	87.8	18.6	53.2
EF02	72.2	18.7	45.5
EF03	79.7	22.0	50.9
EF04	81.7	19.0	50.4
EE05	76.0	18.4	47.2
EE06	36.0	17.8	26.9
EE07	60.1	16.8	38.4
EE08	72.9	16.6	44.8
EC09	81.2	19.9	50.5
EC10	81.4	19.8	50.6
EC11	81.3	19.8	50.6
ET	100	100	100

EF: factorial essay; EE: star essay; EC: essay at the centre; ET1. sample control ET. sample control; Composite powder with respective percentages of *Citrus sinensis* and *Moringa oleifera*: EF01. 77.89/22.11; EF02. 88.14/15.85; EF03. 55.85/44.14; EF04. 65.59/34.4; EE05. 62.5/37.5; EE06. 75/25; EE07. 87.5/12.5; EE08. 58.33/41.66; EC09. 70/30; EC10. 70/30; EC11. 70/30.

4.3. Discussion

Phenolic compounds are secondary metabolites synthesized by plants for protection

against excessive ultraviolet radiation, various physical aggressions or pathogens.

Polyphenolic composition

The total polyphenol contents of the samples are different from those obtained by authors [17]. These authors obtained contents ranging from 24.77 ± 2.02 to 252.65 ± 4.74 mg EAG/g MS through a study carried out on 30 teas from *Camellia sinensis* consumed in China. These differences are due to the species and production technology, as oxidation reduces the total polyphenol content. In addition, polyphenoloxidases transform flavanols into theaflavins and thearubigins, thus reducing polyphenol content [18].

In addition, the herbal teas studied have similar contents to herbal teas from *Chromolaena odorata* [19]. These contain on average 10.61 to 16.02 mg EAG/100mL infusion. Furthermore, authors estimated the total polyphenol content of “Rooibos tea” infusions in South Africa at 25.78 ± 1.12 g EAG/100g [20]. For the same species, obtained 35.07 ± 3.44 mg EAG/100g and 25.05 ± 2.84 mg EAG/100g respectively in methanolic and aqueous extracts (infusion) [21].

Our herbal teas are therefore more concentrated in polyphenols than herbal teas available in these countries. The heterogeneity of the different contents between our herbal teas is partly due to their composition. In addition, the temperature of the water used for infusion, as well as the oxidation time during the production process, has an influence on polyphenol content. Polyphenols are recognized for their health benefits. In fact, they reduce the risk of diseases linked to oxidative stress [22] [23]. In addition, polyphenols are responsible for certain organoleptic properties such as astringency and bitterness in herbal teas [24].

However, certain phenolic compounds such as tannins may reduce the digestibility of food by binding and precipitating dietary carbohydrates, proteins and digestive enzymes. Thus, they are considered antinutrients [25]. In addition, malnourished people may drink our herbal teas moderately, but over-nourished/obese people may drink more. Polyphenols have several health benefits, such as cardiovascular protection and anti-cancer effects, which are associated with their strong antioxidant power. In addition, authors, obtained average total flavonoid contents of 76.82 mg EQ/100g DM in *Moringa oleifera* leaves grown in Côte d'Ivoire [26] [27].

A study showed that *Citrus sinensis* orange peel contained 190 ± 0.09 mg EQ/100 g MS [28]. These contents are low compared with those obtained in our composite herbal teas made from *Citrus sinensis* zest and *Moringa oleifera* leaves. Thus, the combination of the two species would be beneficial in terms of contribution of polyphenolic compounds. The ET infusion (100% *Camellia sinensis*) has a higher total polyphenol and flavonoid content than the various composite teas formulated (EF01, EF02, EF03, EF04, EE05, EE06, EE07, EE08, EC09, EC10, EC11). However, EC09, EC10, EC11, all made up of 70% *Citrus sinensis* and 30% *Moringa oleifera*, have contents closer to the ET control (100% *Camellia sinensis*). In fact, production technology and conditions could have an influence on polyphenol content, as *Camellia sinensis* (ET) tea sold on the market has undergone a well-mastered transformation process.

In a comparative study of several Brazilian teas, [29] obtained flavonoid contents

estimated at 34.09 ± 3.28 mg CTE/L for “*Pimpinella anisum* tea”. These values are lower than those obtained in our composite teas. The latter determined the flavonoid content of “*Camellia sinensis* tea” equivalent to 179.88 ± 32.41 mg CTE/L, higher than our values in composite herbal teas and the ET control consisting of 100% *Camellia sinensis* estimated at 91.26 ± 5.86 mg EQ/L. This difference may be due to the growing area, the tea production technology and the method used for the assay, as our results were determined using a quercetin calibration line.

It is estimated that the average human intake of flavonoids is between 25 mg/day and 1 g/day [30]. Consumption of our herbal teas could therefore cover requirements. Several studies have shown that a diet rich in flavonoids can have beneficial effects on health. Thanks to their ability to inhibit LDL oxidation, flavonoids have significant cardioprotective effects [31] [32]. Another study showed that a high intake of flavonoids can reduce mortality from coronary heart disease (CHD) and lower the risk of CHD by 38% in post-menopausal women [32].

Antioxydant capacity

Several methods can be used to determine the antioxydant activity of extracts: DPPH test, FRAP, ABTS, ORAC. Moreover, the antioxydant activity of extracts cannot be reasonably validated by a single method due to the complex nature of phytochemical compounds and their interactions, hence the importance of using multiple test systems [33] [34]. Thus, in the present study two tests commonly used to assess the antioxydant activity of extracts were employed, namely the DPPH (dpph radical scavenging) and FRAP (Fe^{3+} to Fe^{2+} reduction) assays. These were combined to comprehensively assess the antioxydant activities of composite herbal tea infusions.

The values obtained from the DPPH and FRAP tests show that the infusions have a high level of both antiradical and iron-reducing activity. The antiradical activity of the composite herbal teas, ranging from 122.5 to 298.65 $\mu\text{mol EAA}/100\text{mL}$, and the reducing power (166.92 to 220.63 $\mu\text{mol EAA}/100\text{mL}$) are higher than the averages obtained by Konan *et al.* (2014) in juices consumed in Côte d’Ivoire. The values for the various composite herbal teas are lower than those for tea (*Camellia sinensis*).

In terms of anti-free radical activity, the classification is as follows: ET > EF01 > EF04 > EC10 > EC11 > EC09 > EF03 > EE05 > EE08 > EF02 > EE07 > EE06. FRAP test values indicate the ability of herbal teas to reduce ferric ions (Fe III) to ferrous ions (Fe II). So, in addition to their antiradical activity, the composite herbal teas formulated have a strong reducing power. Among the composite teas formulated, EF03 (55.85% *Citrus sinensis*/44.14% *Moringa oleifera*) has the highest reducing capacity. It is estimated at 220.63 mg AAE/100mL. The reducing capacity of the herbal teas is ranked in ascending order as follows: ET > EF03 > EC11 > EC09 > EC10 > EF04 > EF02 > EF01 > EE05 > EE06 > EE07 > EE08.

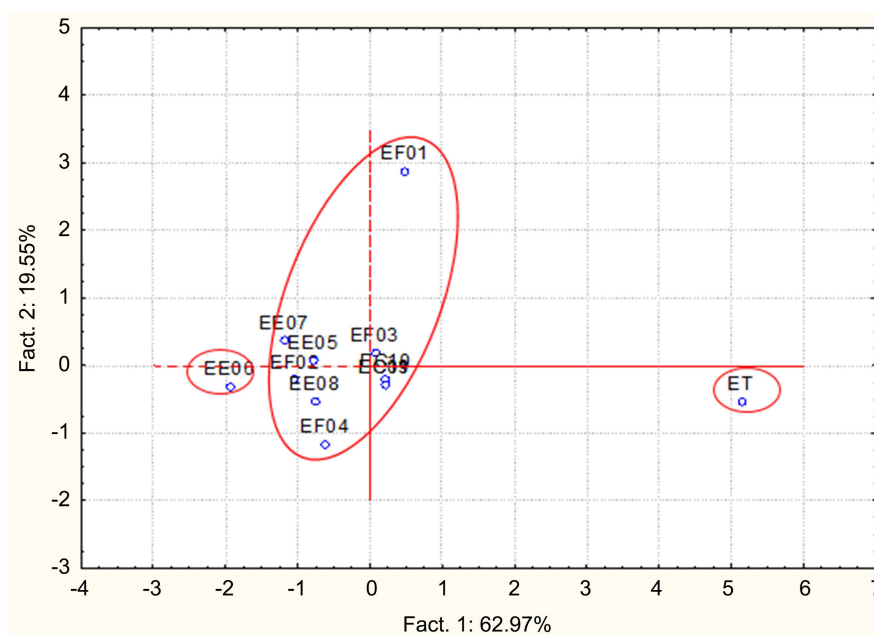
Thus, these herbal teas with their high anti-free radical activity could be beneficial for smokers [35], as antioxydant deficiency is more pronounced in these individuals due to the attacks generated by free radicals in smoke. Elderly (increased oxidative stress with age) and overweight subjects, who constitute a group at risk

of antioxidant deficiency [36], could also benefit from these herbal teas to reduce their deficiency. In addition, for subjects with a high level of potentially prooxidant iron (ferric ions), these herbal teas could play an antioxidant role by reducing these ferric ions to ferrous ions.

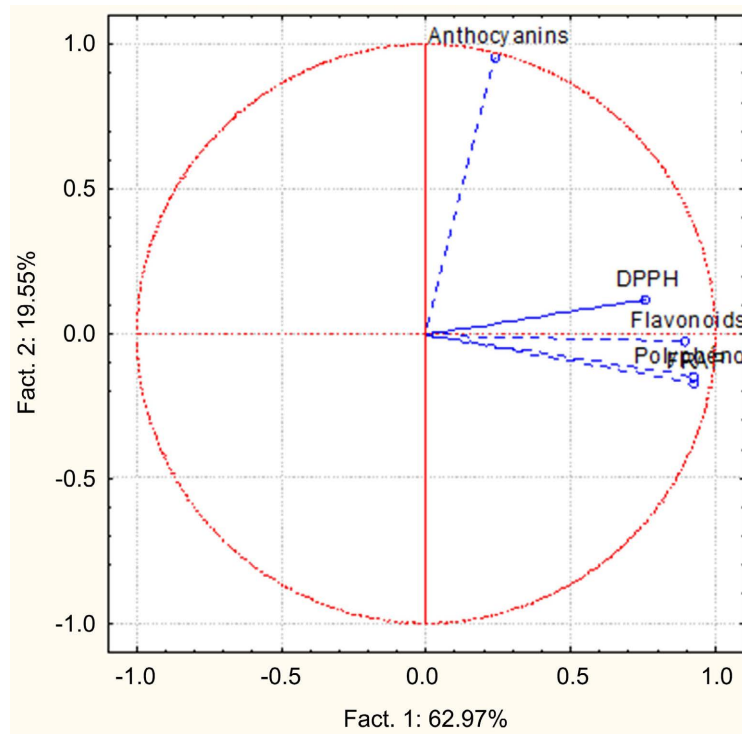
The composite antioxidant index (CIA) of herbal teas is ranked in ascending order as follows: ET > EF01 > EF03 > EC10 > EC11 > EC09 > EF04 > EE05 > EF02 > EE08 > EE07 > EE06. In general, there is a positive correlation between total polyphenol content and composite antioxidant index. Among the composite teas formulated, EF01 (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) has the highest index and the highest total polyphenol content. However, EE06 (75% *Citrus sinensis*/25% *Moringa oleifera*) has the lowest ICA, while EE05 has the lowest total polyphenol content. These results could be explained by a difference in the composition of the herbal teas. In addition, some polyphenols have no free radical scavenging or ferric ion reducing activity [37].

Principal Component Analysis

The quality parameters studied are correlated with 5 factors. However, the F1 factor, with an eigenvalue greater than 1, is used for PCA. It accounts for 62.97% of total variability. Nevertheless, the second factor, with an eigenvalue of 0.97 and total variability of 19.55%, was combined with the first factor for PCA representation. The factor (F1) with an eigenvalue of 3.14 is predominantly formed by all the characteristics studied. These are positively correlated. Parameters and individuals are projected onto the plane formed by factors 1 and 2, which account for 82.52% of total variability (Figure 1 & Figure 2). It divides the individuals into 3 groups. Group 1 is essentially made up of individuals superimposed on traits positively correlated with the F1 factor. They are characterized by high anthocyanin, polyphenol, flavonoid, DPPH and FRAP values.



(a) Projection of individuals



(b) Variable projection

Figure 1. Projection of individuals and herbal tea variables studied in factorial plane 1 - 2 of principal component analysis.

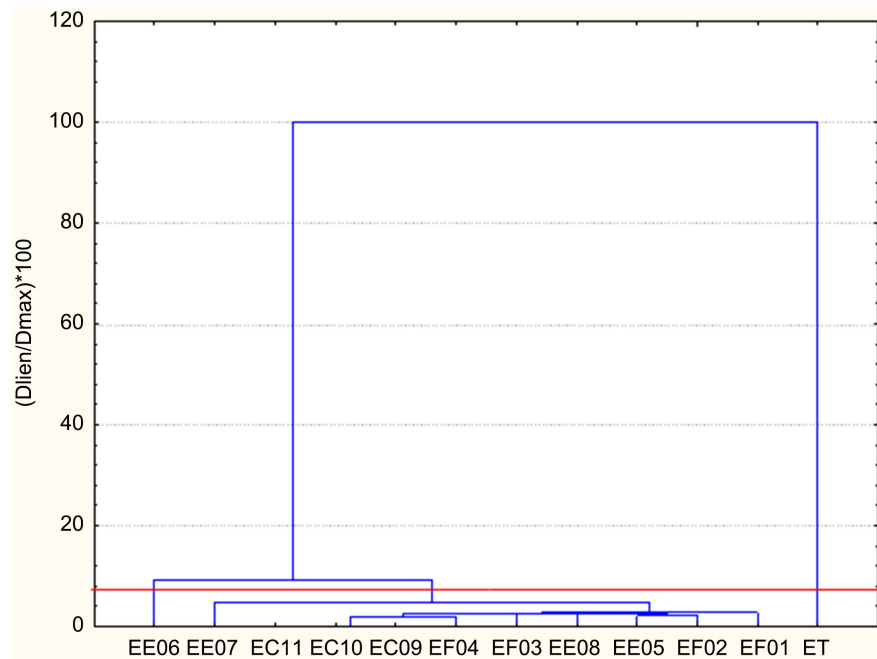


Figure 2. Hierarchical classification of herbal teas according to polyphenolic characteristics.

This is the ET sample. The second group contains samples with lower values than those in the first group. Samples in the third group are characterized by low

values for anthocyanins, polyphenols, flavonoids, DPPH and FRAP.

Hierarchical ascending classification (HAC) using the Euclidean distance method confirms the variability observed in PCA. Truncation of the dendrogram at an aggregation Euclidean distance of 8 reveals three classes (**Figure 2**). Individuals in the first class are distinguished by higher anthocyanin, polyphenol, flavonoid, DPPH and FRAP values than the other samples analyzed. The second class represents the intermediate class, distinguished by anthocyanin, polyphenol, flavonoid, DPPH and FRAP values lower than those of the first class, but higher than those of the third class. The third class includes samples with low anthocyanin, polyphenol, flavonoid, DPPH and FRAP values.

5. Conclusion

The aim of this study was to determine the polyphenolic composition of the herbal teas and assess their antioxidant activity. The results provide a database for the production of herbal teas with good polyphenolic and antioxidant properties from orange peel (*Citrus sinensis*) and *Moringa oleifera*. EF01 (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) had the highest polyphenol content, while EC10 (70% *Citrus sinensis*/30% *Moringa oleifera*) had the highest flavonoid content. EF01 (77.89% *Citrus sinensis*/22.11% *Moringa oleifera*) has the highest reducing activity, while EF01 has the highest anti-free radical activity. There is a strong correlation between polyphenol content and the anti-free radical and reducing activity of herbal teas.

Conflicts of Interest

Authors have declared that no competing interests exist.

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