



Phytochemical Characterization of Herbal Tea from Oranges Peels (*Citrus sinensis* var *Blonde*) Marketed in Abidjan

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Authors' contributions

This work was carried out in collaboration among all authors. Author ARREY supervised the entire study. Author KKA designed the study, performed the experiment, carried out the statistical analysis and wrote the manuscript. Author FNBY monitored the performance of the experiment. Author FNBY edited the manuscript. All authors have read and approved the final manuscript.

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ABSTRACT

Sweet orange (*Citrus sinensis* L.) is the most widely consumed citrus fruit in Côte d'Ivoire. They are rich in bioactive compound. Also, their peels or flavedo rejected during consumption have a high content of polyphenols, flavonoids, and essential oils. However, in Côte D'Ivoire, studies are limited to the characterization of orange peel (*Citrus sinensis*). Our study therefore aims to determine the phytochemical composition of herbal teas obtained from the orange peel (*Citrus sinensis*) in order to contribute to their valorisation as herbal teas. The *Citrus sinensis* zests were collected in Abidjan (Côte d'Ivoire) in Adjamé community. An herbal tea was produced and subjected to phytochemical and physicochemical tests. The results showed that the moisture content of the different batches varies from 14.13±0.41 to 7.2%±0.2; the oven-dried batches showed the lowest value. In addition, the ash content remains statistically different between batches. The herbal teas studied had an

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average ash content of 6.53%. The pH values negatively correlated to acidity range from 3.92 ± 0.2 to 3.21 ± 0.15 . Also, these herbal teas contain several secondary metabolites such as polyphenols, flavonoids, alkaloids, tannins and saponins. This study shows that herbal teas made from orange peel contain several phytochemicals including antioxidants. In addition, their high ash content suggests a high mineral content. Therefore, orange peels can be used in the form of herbal teas as a dietary supplement.

Keywords: Oranges; *Citrus sinensis*; herbal tea; antioxidant; Côte d'Ivoire.

1. INTRODUCTION

The largest world's fruit provision is citrus with more than 110 million tons in 2016 [1]. These citrus fruits include lemons, mandarins, pomelos, citrons, oranges, grapefruits and limes [2]. Among them, sweet oranges are the most consumed because of their good flavour, nutritional value and composition of biological active molecules [3].

In Côte d'Ivoire, the production of oranges is estimated at 42,000 T/year for an area of 5,000 ha. Most of this production is marketed through informal channels. Purchased oranges are generally peeled and then consumed by pressing the juice or processed into nectar. This, generates a large quantity of by-products which are the peel or epicarp, the mesocarp, the endocarp and the seeds. The peel, also called flavedo or zest, is the outer part of citrus fruit. Different authors have shown their richness in secondary metabolites such as flavonoids, terpenes and essential oils. These compound are characterized by their therapeutic, antioxidant, antiviral, antifungal and antibacterial activity [4-5]. In addition, zest is used to fight cardiovascular disease, also as an insecticide and nutraceutical [6]. A group of flavonoids, polymethoxyflavones (PMFs), which is found abundantly and almost only from the citrus peels, have been given great attention because of their wide range of properties. Many in vitro experiments elucidated anticancer actions by PMFs such as antiproliferation, enzyme inhibition and cancer cell growth inhibition [7]. Various potent antioxidants have been found in citrus peels and showed antioxidant effects including free radical scavenging and metal chelation activities [8].

However, in Côte d'Ivoire, this zest is a consumer waste and thus a source of environmental pollution. In addition, very few studies have been carried out on *Citrus sinensis* peel. In addition, the removal of peels is generally limited to, the purpose of extraction of essential oils [9]. Thus, new ways of recovery

such as the production of herbal teas would be beneficial to the orange sector in the Ivorian population given the high content of phytochemicals indicating the antioxidant activities and therapeutic effect of *Citrus sinensis* peel. Indeed, citrus peel or leaf infusion has long been used in the medical system for therapeutic purposes, particularly for the treatment of colds and diarrhoea [6].

Thus, the present study aims to determine the phytochemical composition of herbal teas, made from *Citrus sinensis* peel. For this purpose, peel (zests) bought in the market of Adjamé (Abidjan, Côte d'Ivoire) will be used to prepare the herbal teas from which the major chemical compound will be identified.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Plant material

The plant material consisted of zests also called flavedo or epicarp of mature oranges (*Citrus sinensis* L.) with yellow-green epidermis.

2.1.2 Technical material

Weighings were performed on a weighing scale (shimadzu, Japan) and a precision weighing balance (OSI, M-220 D). Also, a binatone-brand blender (BLG 555, China) was used to grind the peel and then sieved using commercial sieves (mesh size $\leq 100 \mu\text{m}$). Moisture was determined using an oven (Memmert UL 30, Germany). A Nabertherm muffle furnace (D. 2804, Bremen, Germany) was used to determine the ash content. In addition, beakers, a spatula, a balloon, a sand bath were used.

2.1.3 Chemical material

All solvents and chemical reagents used in this study are analytical grade. The colour indicator phenolphthalein, sodium hydroxide was supplied

by Sigma-Aldrich (Germany). The following solvents: absolute ethanol, methanol; isoamyl alcohol, hydrochloric alcohol supplied by VWR Chemicals (France) were used. Dragendorff (potassium iodobismuthate reagent) (Sigma-Aldrich, France), Stiasny (hydrochloric formaldehyde solution), Liebermann (acetic anhydride and sulphuric acid), and ammonia (Sigma-Aldrich, India) reagents were used for phytochemical screening.

2.2 Methods

2.2.1 Study zone

The samples were collected in the municipality of Adjamé located in Abidjan city in the southern part of Côte d'Ivoire. This market is the largest market for citrus fruits, especially oranges, where all orange wholesalers and traders in Abidjan receive their supply [9]. Samples of orange peel were collected from orange sellers at the four orange supply sites in this market.

2.2.2 Sampling

Citrus sinensis peels were collected from orange sellers at 4 sites of the wholesale market of Adjamé. At each site, 1 kg of peel was collected from each of the three randomly selected female sedentary orange traders. Subsequently, a pool was made per site from which 4 batches of 3 kg of peel were obtained. After collection, the samples were taken to the Laboratory for zest treatment. Analyses were carried out jointly at the Laboratory of Biochemistry and Food Sciences, Laboratory of Botanical Pharmacognosy, Faculty of Pharmacy and Biological Sciences of Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire

2.2.3 Zests treatment

Citrus sinensis peel was treated according to the method described by Ekissi [10] modified.

The 4 batches of *Citrus sinensis* zests collected were sorted and the waste discarded, then washed rapidly with potable water and then dried to reduce the microbial load. Afterwards, these clean organs were shrivelled in an oven at 50°C for 6 hrs. After cooling they were oxidized, then each batch was subdivided into three sub batches. This was followed by drying the first sub-batch in the oven at 50°C, the second was dried at 20°C and the third in the sun (28-31°C). The dried peel was then subjected to grinding till

become powder. The grinding and sieving process was repeated.

2.2.4 Preparation of herbal teas

For this preparation, 100 ml of boiling water was poured over 2 g of coarse powder of *Citrus sinensis* peel. This mixture, in a closed container, was uncovered, from time to time, to be stirred with a glass rod until completely cooled after 5 min. The filtrate was recovered after filtration of the mixture on Whatman paper.

2.2.5 Determination of humidity of powder

The moisture content was determined according to the AOAC [11] method. It consisted of placing 5 g of samples from each batch contained in a capsule in an oven (MEMMERT) and set at 105°C to constant weight.

2.2.6 Détermination of ash content in powder

Ash content was determined according to AOAC [11] method. Thus, 5 g of dried samples were placed in a Naberthern muffle furnace (Germany) and then incinerated at $550 \pm 15^\circ\text{C}$ for 12 hours.

2.2.7 Determination of pH and titratable acidity

These parameters were determined using the AOAC [11] method. A volume of 10 ml of herbal tea was used to determine the pH using a previously calibrated Hanna pH meter (HI 9124). For the determination of the titratable acidity the same solution was titrated with a 0.1 N NaOH solution after the addition of three drops of phenolphthalein.

2.2.8 Phytochemical study

The detection of the presence of certain secondary metabolites (polyphenols, flavonoids, alkaloids, saponins and terpenes) in the extracts was carried out according to the method described by [12]. The presence of a secondary metabolite was determined by observing the expected coloration.

2.2.8.1 Search for total polyphenols

Total polyphenols in the herbal teas was detected by adding a drop of 2% FeCl_3 aqueous solution to 2 mL. The presence of phenolic compound was detected by the appearance of a

more or less dark blue-blackish or green coloration.

2.2.8.2 Flavonoid detection

Identification of total flavonoids was carried out by evaporating 2 mL of herbal tea on a sand bath. After cooling, the dry residue was taken up again in 5 mL of hydrochloric alcohol. This solvent is a mixture of 96° ethanol, distilled water and 12.1 N (v/v) hydrochloric acid. Then 2 to 3 magnesium chips were added. The presence of total flavonoids was revealed by the appearance of a pinkish-orange coloration after the addition of 3 drops of isoamyl alcohol.

2.2.8.3 Search for alkaloids

The detection of alkaloids in the herbal tea was performed by evaporating 6 mL of herbal tea on a sand bath to dryness. Two drops of dragendorff reagent were added to the residue in 6 mL of ethanol (60%). The presence of alkaloids was revealed by the appearance of an orange precipitate.

2.2.8.4 Search for tannins

Tannin determination was performed by dissolving the dry extract of 5 mL of infusate in 15 mL of Stiasny's reagent. This solution was incubated in a water bath at 80°C for 30 minutes. The presence of catechic tannins was revealed by the appearance of flakes, precipitating slowly. The solution containing the flakes was then filtered. This filtrate was saturated with sodium acetate. The presence of gallic tannins was revealed by the appearance of a blue-black coloration after the addition of 3 drops of 2% FeCl₃.

2.2.8.5 Search for saponins

To identify the saponins, an aliquot of 10 mL of herbal tea was placed in a test tube. After manually shaking the tube for a few minutes, the height of the foam was measured. The presence of saponins was detected by the presence of a foam height greater than 1 cm on the surface of the extract.

2.2.8.6 Search for sterols and terpenes

An aliquot of 10 mL of herbal tea was mixed with 1 mL of acetic anhydride and 1 mL of chloroform. After gentle shaking, the resulting solution was divided by volume into two test tubes, one of which was used as a control. The test tube received 1 mL of concentrated sulfuric acid. At

the interface, a brownish-red or purple ring was formed. The presence of sterols and terpenes was revealed by turning the supernatant green.

2.3 Statistical Analysis

All analyses were performed in triplicate and the data obtained was recorded in an Excel file. The statistical analysis of the data was carried out using Statistica 7.1 software. The means of each parameter determined for each sub-batch were compared by an ANOVA analysis of variance to a factor, followed by a Newman Keuls post-test at the 1% significance level.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Physicochemical characteristics of powder of treated *Citrus sinensis* peel

The results in Tables 1 and 2 show the physicochemical characteristics of *Citrus sinensis* peel powder and herbal teas, respectively.

3.1.1.1 Humidity

The moisture content of the treated zest powder is significantly different at $p < .01$ depending on the drying method. Oven-dried batches contained the lowest moisture content. These values are between 7.20 and 12.80% (Table 1).

3.1.1.2 Ashes

In general, the ash contents obtained are not statistically different. The extremes (highest and lowest), significantly different contents were respectively observed with batches C2 (6.53%) dried at 20°C and S4 (5.26%) sun-dried (Table 1).

3.1.2 Physicochemical characteristics of herbal teas made from treated *Citrus sinensis* peel

3.1.2.1 pH

The pH values range from 3.21 ± 0.15 to 3.93 ± 0.56 . Oven-dried lot 4 (E 4) has the lowest pH with its pH = 3.2 (Table 2).

3.1.2.2 Titratable acidity

The acidity varies from 0.60 to 1 meq/100g. There is a statistical difference between the acidity of the different samples (Table 2).

Table 1. Physicochemical parameters of treated *Citrus sinensis* peel

batches		Moisture (g/100g)	Ashes (mg/100g)
Lot 1	S1	12.86 ^c ± 0.30	6.06 ^b ± 0.11
	C1	14.13 ^a ± 0.41	6.13 ^b ± 0.30
	E1	12.80 ^c ± 0.20	6.00 ^b ± 0.20
Lot 2	S2	8.80 ^e ± 0.21	6.06 ^b ± 0.23
	C2	13.80 ^a ± 0.20	6.53 ^a ± 0.11
	E2	7.20 ^h ± 0.20	6.46 ^a ± 0.23
Lot 3	S3	9.20 ^e ± 0.34	5.86 ^b ± 0.11
	C3	14.40 ^a ± 0.20	6.06 ^b ± 0.11
	E3	12.06 ^d ± 0.23	5.93 ^b ± 0.11
Lot 4	S4	12.46 ^c ± 0.46	5.26 ^c ± 0.11
	C4	13.53 ^b ± 0.50	5.73 ^b ± 0.11
	E4	8.40 ^g ± 0.36	6.00 ^b ± 0.11

S: sun-dried batch (28-31°C); C: air-dried batch (20°C); E: oven-dried batch (50°C). Averages with the same letters in the same column are not significantly different at 1% according to the Newman Keuls test ($p < .01$)

Table 2. Physicochemical parameters of *Citrus sinensis* peel herbal teas

batches		pH	Titratable acidity (meq/100g)
Lot 1	S1	3.66 ^c ± 0.08	0.83 ^a ± 0.15
	C1	3.88 ^b ± 0.05	0.66 ^b ± 0.11
	E1	3.23 ^f ± 0.12	1.00 ^a ± 0.11
Lot 2	S2	3.55 ^d ± 0.04	0.86 ^a ± 0.11
	C2	3.88 ^b ± 0.20	0.60 ^c ± 0.00
	E2	3.56 ^d ± 0.20	0.66 ^b ± 0.05
Lot 3	S3	3.66 ^c ± 0.05	0.73 ^b ± 0.05
	C3	3.38 ^e ± 0.07	0.80 ^a ± 0.15
	E3	3.92 ^a ± 0.03	1.00 ^a ± 0.13
Lot 4	S4	3.93 ^a ± 0.06	0.80 ^a ± 0.21
	C4	3.85 ^b ± 0.46	0.66 ^b ± 0.11
	E4	3.21 ^f ± 0.15	1.00 ^a ± 0.20

S: sun-dried batch (28-31°C); C: air-dried batch (20°C); E: oven-dried batch (50°C). Averages with the same letters in the same column are not significantly different at 1% according to the Newman Keuls test ($p < .01$)

3.1.3 Influence of the drying method on the physicochemical characteristics of powder of orange peel

Fig. 1 shows the influence of the drying method on the physicochemical parameters of moisture and ash of treated *Citrus sinensis* peel powders. Moisture and ash contents of oven-dried and

sun-dried batches are not statistically different. However, the moisture content (14%) of the orange peel powder obtained by drying at 20°C under air conditioning is higher. The lowest values (10.11%) were observed in oven drying. The ash proportions are statistically identical to a higher algebraic value for oven-dried peel (6.1%).

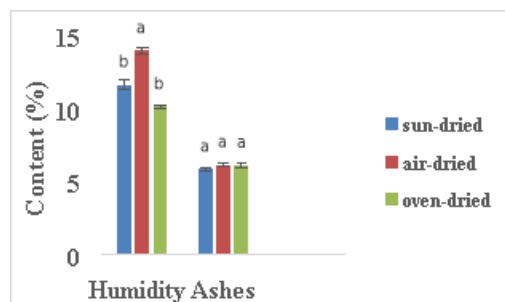


Fig. 1. Influence of the drying method on the physicochemical parameters of powder of treated *Citrus sinensis* peel

3.1.4 Influence of the drying method on the physicochemical characteristics of herbal tea made from *Citrus sinensis* peel

Fig. 2 shows the influence of the drying methods (sun drying, air conditioning, and oven drying) on the pH of herbal teas. The pH reflects the acidity of the herbal tea regardless of the drying method. These values range from 3.48 to 3.7.

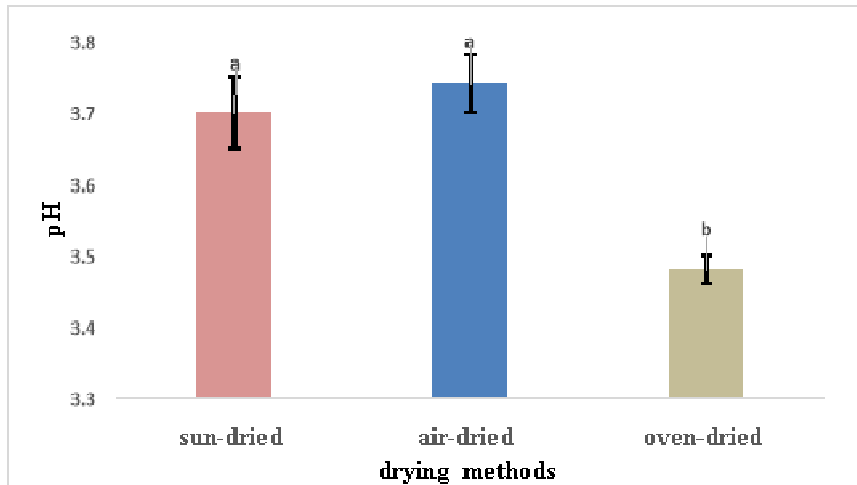


Fig. 2. Influence of the drying methods on the pH of herbal tea from *Citrus sinensis* peel

The method of drying (drying in the sun, under air conditioning, and in the oven) has an influence on the titratable acidity of the herbal teas. Oven and sun-dried batches have the highest titratable acidity values (Fig. 3). These contents are statistically different from those dried at 20°C.

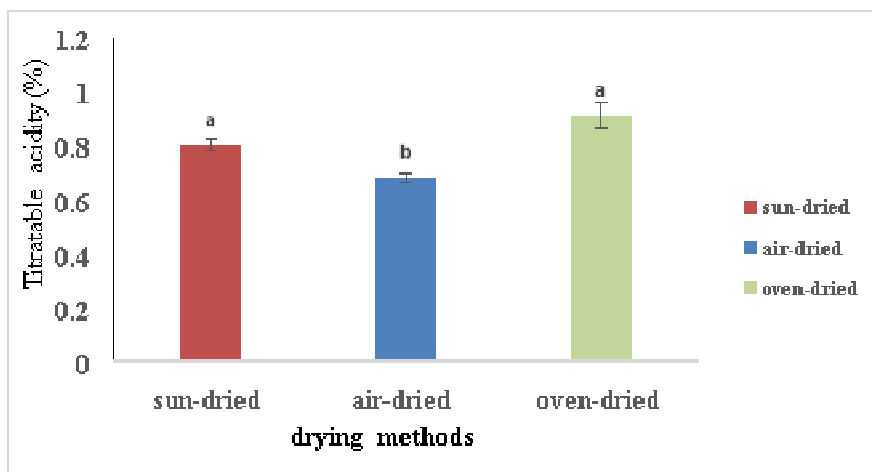


Fig. 3. Influence of the drying method on the titratable acidity of the herbal tea from *Citrus sinensis* peel

3.1.5 Phytochemical characteristics of herbal teas

The results of the phytochemical tests of the herbal tea are recorded in Table 3. The phytochemical study of herbal teas from *Citrus sinensis* zest (flavedo) revealed the existence of several secondary metabolites

which are total polyphenols, flavonoids, alkaloids, tannins and saponins. Thus, polyphenols and terpenes are present in all herbal teas. As for flavonoids, they are not detected in all herbal teas from the same batch. In addition, certain chemical group (alkaloids, tannins and saponins) are not detected in all herbal teas.

Table 3. Phytochemical screening of herbal tea from *Citrus sinensis* peel

	Herbal tea	Pph	Flav	Alc	Tan C	Tan G	Sapo	Stér et Terp
Lot 1	S1	+	-	+	-	-	+	+
	C1	+	+	+	+	-	+	+
	E1	+	-	+	+	-	+	+
Lot 2	S2	+	+	-	-	-	+	+
	C2	+	+	+	-	-	+	+
	E2	+	+	+	+	+	+	+
Lot 3	S3	+	-	+	+	-	-	+
	C3	+	-	+	-	-	+	+
	E3	+	+	-	+	-	+	+
Lot 4	S4	+	+	+	-	-	-	+
	C4	+	+	+	+	+	+	+
	E4	+	-	-	+	-	+	+

S: sun-dried batch (28-31°C); C: air-dried batch (20°C); E: oven-dried batch (50°C). Pph: Polyphenols; Flav: Flavonoids, Alc: Alkaloids; Tan C: Catechic tannins; Tan G: Gallic tannins; Sapo: Saponins; Ster and Terp: Sterols and terpenes+; Presence, -: Absence

3.2 Discussion

The results of physicochemical analyses showed that the moisture content of powder resulting from drying at 20°C is higher than 12%. However, those dried in the oven vary from 7.2 to 12.8 %, which is very close to those dried in the sun. Indeed, the moisture content is an indicator of good preservation. Its maximum limit for good preservation of foodstuffs is 12% [13]. The results indicate that the sun-dried and oven-dried lots are close to this limit. These results correspond to those obtained by Okafor *et al.* [14] in *Moringa oleifera* tea (12.62±0.07). Indeed, drying in the oven allowed the maximum amount of water to evaporate from the powders. This is the water of constitution, due to the high temperature. Only the bound water remains, which can only be removed at 130 °C. Thus, the herbal teas resulting from the drying of orange peel in the oven can be stored longer, because of their low moisture content. In addition, the shelf life of food depends on certain intrinsic factors, including the activity of water (Aw), responsible for their alterations [14]. The control of moisture content in herbal tea and tea products is an important factor in preservation, especially for the inhibition of microbial growth [15].

Ash content is an indicator of good mineral composition. The highest value was obtained in C2 (6.53% ±0.11). This indicates that *citrus sinensis* powder would be a good source of minerals. Indeed, ash is a residue of mineral compounds that remains after the incineration of a sample containing organic substances of animal and plant origin. These rates are closer to

those obtained by Falade *et al.* [16] who worked respectively on *Camellia sinensis* and *Hibiscus sabdariffa*, but below those obtained by Ekissi [10] on *Lippia multiflora*. The differences in our different samples may be due to soil conditions as the minerals are taken directly from the soil by root absorption of the plant.

The pH of herbal teas was between 3.21 and 3.92 which reflects their acidity. These pH values are approximately equal to those obtained by Flores-Martinez *et al.* [17] in a study of *Camellia sinensis* tea of different flavours with values ranging from 3.82 to 4.11. Furthermore, these acidic pH values would inhibit the proliferation of microorganisms in herbal teas. The parameters pH and titrable acidity are used to estimate the quality of the products. Indeed, the pH measures the content of strong acids in a medium such as acids and minerals. However, titratable acidity combines the contents of strong acids and those of weak acids represented by volatile organic acids such as fatty acids, amino acids and CO₂ [18-19].

The beneficial health effects of bioactive compounds in plants are generally due to polyphenols known for their strong antioxidant activity. Terpenic compound is present in all herbal teas. In addition, they are the source of its aroma [20]. These secondary metabolites would be responsible for the antioxidant and stimulating properties due to the respective presence of flavonoids and alkaloids. They also have astringent, colouring virtues related to the presence of tannins, surfactants due to saponins [21].

Several studies have confirmed the therapeutic activity attributed to the polyphenols of orange peel from *Citrus sinensis*, including their antioxidant, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic properties [22-23]. They are capable of trapping reactive oxygen species formed in response to environmental aggressions (tobacco smoke, UV rays, pesticides, pollutants, etc.) or waste generated by the organism during metabolic processes [24]. Indeed, they have an ideal chemical structure capable of fighting free radicals due to their large number of hydroxyl groups. Thus, studies *in vitro* have shown that their antioxidant capacity is stronger than that of vitamins C, E and carotenoids [25].

Moreover, our study reveals the presence of flavonoids in most samples. However, they are not observed in some extracts unlike those of Wang *et al.* [26]. This would be due to the identification method, which is a qualitative method. Thus, a low level might not be observed.

Catechic tannins have been observed in some herbal tea extracts (C1, E1, E2, S3, E3, C4, E4). This chemical group is known for its multiple health benefits, including anti-diabetic and anti-cancer properties [27].

Studies conducted by Ekissi [10] and Okafor *et al.* [14] have shown the presence of tannins in *Lippia multiflora* and *Moringa oleifera* herbal teas. Indeed, these compounds are responsible for the astringency of the herbal teas. They are involved in the treatment of inflamed or ulcerated tissues [28-30]. The presence of these polyphenolic compounds in herbal teas justified the traditional medicinal use of orange peel in the treatment of certain infections such as abdominal pain and influenza.

Alkaloids are generally observed in the herbal teas studied, as in the tea of *Camellia sinensis* in the work of Salawu *et al.* [20]. Several biological properties are attributed to this chemical group. They are antihypertensive and stimulant properties due to certain alkaloids such as caffeine, theobromine. Thus, they are able to reduce migraines associated with hypertension. On the other hand, certain alkaloids such as cocaine and mescaline are considered as antinutrients because of their action on the nervous system. These are synthesized by plants to control pests. Indeed, in some areas, the aggregation of plants and the low diversity exposes plants to insect pests.

Saponins have been observed in most herbal teas. This chemical group was also observed in the *Lippia multiflora* herbal teas studied by Ekissi [10]. Saponins are heterosides called "natural detergents" because of their foam. Saponin-rich leaves such as those of *Moringa oleifera* are used as soap by some communities [30]. Saponins have anticancer properties, modulating immune and regulatory activities and health benefits such as inhibiting the growth of cancer cells.

Differences in physicochemical and phytochemical characteristics have generally been observed within the same batch of differently dried herbal teas. The drying temperature would have an influence on both the physicochemical and phytochemical composition, as well as the stage of ripeness, the origin of the fruit (the climate, the nature of the soil, growing conditions or the application of natural or artificial fertilizer on the orange trees) would be at the origin of these differences because even if a mixture was made after collection, it may not be homogeneous. In addition, the multiplicity of cultivars within the same variety (blond) would be at the origin of this heterogeneity of the results of the same batch of herbal teas.

4. CONCLUSION

The objective of this study was to determine the phytochemical composition of herbal tea made from *Citrus sinensis* peel. The results provide a basis for the production of herbal teas of good nutritional quality from *Citrus sinensis* peel. The low moisture content of the oven-dried peel powder (10.11 ± 3.07) shows that they could be preserved for a long term if well packaged. In addition, their high ash content (6.11% DM) suggests high mineral content. Moreover, the presence of phytochemical compounds such as polyphenols, flavonoids and catechic tannins show that herbal teas made from *Citrus sinensis* peel are interesting antioxidant compounds. Overall, the different drying modes do not influence the presence of phytochemicals compounds. Thus, orange peel can be used in the form of herbal teas. However, the identification of phytochemical compounds and the determination of the biochemical characteristics of the herbal teas need to be carried out. Taking into account all these factors would allow a better characterization of the herbal tea made from *Citrus sinensis* peel, in order to enhance their nutritional value.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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